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Reproductive biology and biotechnologies in wild felids

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ABSTRACT

Conservation strategies in natural habitats as well as in breeding centers are necessary for maintaining and reinforcing viable populations of wild felids. Among the fundamental knowledge that is required for conservation breeding, a solid understanding of reproductive biology is critical for improving natural breeding and enhance genetic diversity. Additionally, it offers the opportunity to develop assisted reproductive technologies (ARTs) in threatened and endangered species. Conservation breeding and reproductive biotechnologies of wild felids have advanced in the past decade. It has been clearly shown that female felids have species and individual patterns of reproductive cycles and respond differently to exogenous hormones. In males, several species still have poor semen quality often due to the loss of genetic diversity in small populations. To overcome the challenges of natural breeding (incompatibility between individuals or suboptimal environment) and mitigate inbreeding, artificial insemination, embryo production and embryo transfer have been further developed in 24 wild cat species. Major factors limiting ART success are inconsistent responses to ovarian stimulation, variable quality of gametes and embryos, and preparation of recipient females. Additional approaches including stem cell technologies have been explored for future medical applications. However, there still is a critical need for better knowledge of feline reproductive biology and improvement of ARTs efficiency to increase the genetic diversity and create sustainable populations of wild felids.

1. Introduction

Most of the 38 species in the Felidae family, except the domestic cat are classified as threatened, vulnerable or endangered because of illegal hunting, habitat loss and degradation. Conservation strategies, e.g., habitat management and protection, translocation, captive breeding and reproductive technology, are necessary for maintaining their genetic diversity [1]. *Ex situ* reproductive research programs to establish genome resource bank, produce offspring and reintroduce endangered species back to the wild are essential to strengthen the *in situ* conservation. Although natural breeding is the first reproductive management of choice for sustaining the endangered wildlife populations in captivity, many of them cannot reproduce well due to various reasons, e.g., male aggression, behavioral incompatibility and infertility. Consequently, intensive captive breeding management using assisted reproductive technologies (ARTs), i.e. artificial insemination (AI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), somatic cell nuclear transfer (SCNT) and induced pluripotency stem cell (IPSC) have been explored to help expanding the captive populations. However, basic reproductive biology is mandatory, being the first step to support both natural breeding management and ARTs. For instance, the Zoological Park Organization (ZPO) in Thailand has long been working in collaboration with local universities and international organizations to establish captive breeding, research laboratories, cryobank and ARTs in wild cats since 1993. Cryobanking of gametes, embryos and somatic cells of nine endemic endangered felid species have been established at ZPO aiming at maintaining their genetic diversity and producing genetically desired offspring. Semen analysis and cryopreservation

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are currently readily available for research and routine AI and IVF activities. For the cell bank, tissues of endangered felids collected during annual physical examination or from fresh carcasses are routinely cultured and maintained as cell lines for SCNT and IPSC (Fig. 1).

In addition, zoo animal populations around the world are critical to a common goal of conserving species. This is an invaluable asset, not only for education and outreach, but also providing access for researchers to study species and collect data. Integration of reproductive biotechnology into health management programs in zoo populations would be of great benefit for felid conservation. The purpose of this review is to gather basic and applied knowledge about felid species *ex situ* collected over the past years to protect them from extinction.

2. Reproductive biology

Apart from the domestic cats (*Felis catus*) whose gonads are accessible from veterinary spay/neuter clinics as disposals, reproductive biology studies are performed in zoo-based populations with limited numbers of samples. The common ovarian steroid hormone pattern in felids is characterized by wide range estrous cycle and the seasonality of reproduction varies between cat species [2]. Semen collection requires anesthesia and restraint that are the major constraints to collect thorough informative data in the zoo animals. For hormone studies, sex steroids in wild animals are non-invasively measured from feces [3] and saliva [4]. In males, apart from electroejaculation, wild cat sperm are obtained post-mortem from epididymides and testes.

2.1. Reproductive biology in the female

The domestic cats are seasonally long-day breeders. However, under a natural temperate photoperiod, they undergo estrous cycles throughout the year without a long ovarian paucity [5–7]. Follicular growth is usually detected by an abrupt overt signs of sexual behavior, e.g. rubbing, lordosis, attracting to males, and tail raised. Unlike the domestic cats, many wild felid species in captivity rarely display overt signs of sexual receptivity (e.g. clouded leopard; *Neofelis nebulosa*) [8]. However, vocalization and rolling are presented as the major estrus behavioral in the Asiatic lions (*Panthera leo persica*) [9]. The duration of estrus appears similar in all felid species, except that longer periods of estrus may be observed in the domestic cats, sand cats (*Felis margarita*), rusty-spotted cats (*Prionailurus rubiginosus*), jaguars (*Panthera onca*), snow leopards (*Panthera uncia*) and clouded leopards [10]. Vaginal cytology and ovarian ultrasonography can be performed in domestic cats to confirm estrus as well as to monitor continuous ovarian activity, respectively. Unfortunately, these methods are not feasible in wild felids without anesthesia. In addition, when AI is used in non-domestic felids, estrus observation is not always necessary because estrus and ovulation inductions are performed at fixed time according to the hormonal induction regimens. For example, administration of hCG is recommended at 80–84 h after eCG [11] to expect the ovulation consequence at 25–33 h [12].

Estrus patterns in felids range from reflex LH release by vaginal stimuli during mating (induced ovulation) to endogenous steroid-induced LH surges (spontaneous ovulation). With unknown triggering mechanism, the felids exhibit inconsistent estrus patterns

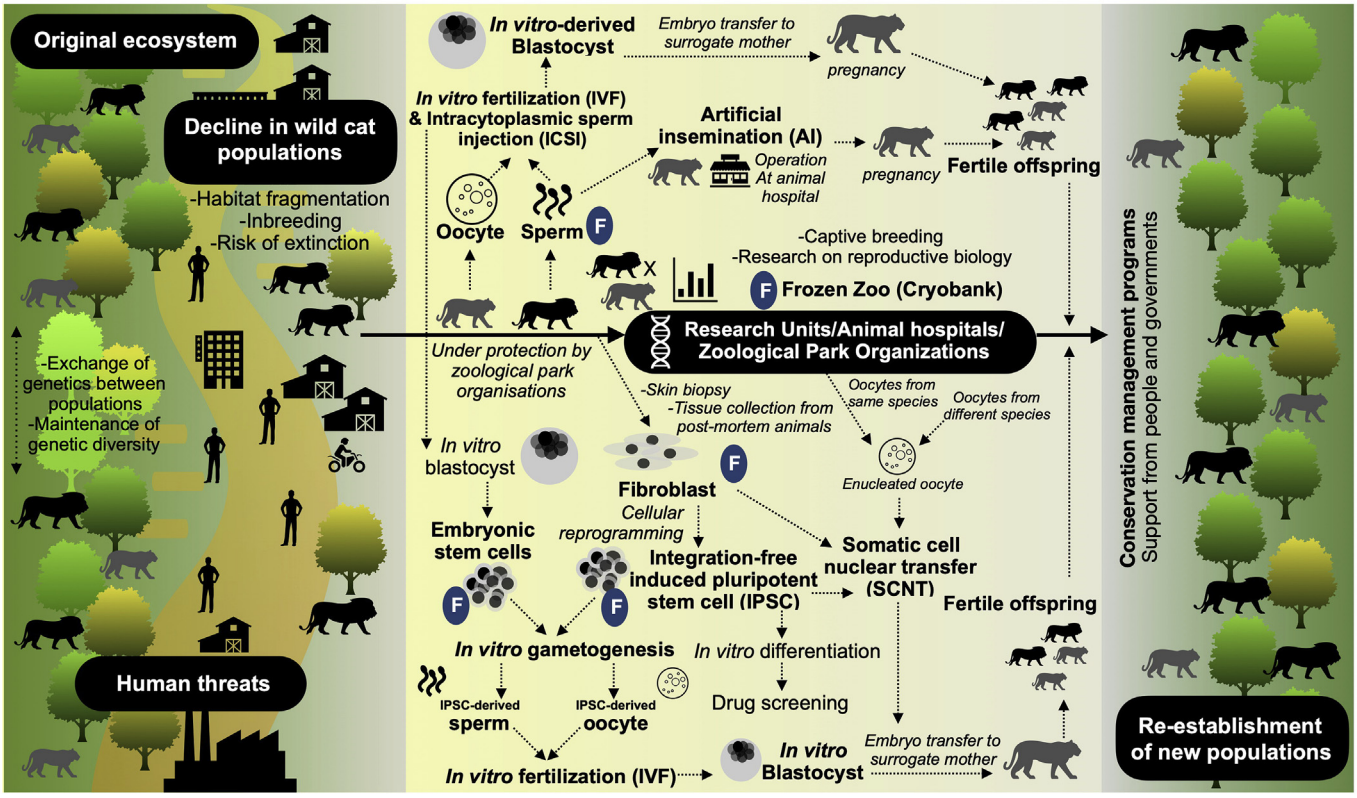

Fig. 1. Overview strategies of using reproductive and cell technologies to save wild cat species from extinction. Human threats worldwide lead to population decline of wild cats. Zoological Park Organization plays central role in gathering teams of veterinarians and research scientists to save wild cats via reproductive technology including *in vitro* fertilization (IVF), artificial insemination (AI), embryo transfer (ET) and intracytoplasmic sperm injection (ICSI) and via cell technology including somatic cell nuclear transfer (SCNT), embryonic stem cells (ESCs) derivation and generation of induced pluripotent stem cell (IPSCs). The ZPO also aims to establish "Biobank of frozen cells (F)" including sperms, oocytes, embryos, ESCs and IPSCs, which provides long-term preservation of good genetics of wild cat species and prevent them from extinction.

varying among individuals and among estrus cycles in the same females. Spontaneous ovulation occurs up to 60% of the domestic cats [13] and 43% of the clouded leopards [14]. Tigers (*Panthera tigris*) are primarily induced ovulators because fecal progestagens increase only after breeding [15]. In general, estrogen positive feedback is a key regulator to induce a gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) surge leading to spontaneous ovulation [16] whereas mating stimuli is required to induce ovulation via the brain control in the induced ovulators. In the past, coitus-induced ovulation is suggested to occur in women [17]. Moreover, the rats show reflex ovulation when maintained in a constant-light condition [18]. From the above reports, mammalian species may have an innate mechanism for reflex ovulation that is likely a more ancient form of reproduction than spontaneous ovulation [19]. These inconsistent estrus patterns bring us a question whether the spontaneous ovulators have an inherent mechanism mediating mating-induced ovulation [19]. Understanding of the evolutionary theory might help finding the inherent central mechanism regulating the inconsistent estrous patterns among the felid species. Recent reproductive neurosciences indicate that kisspeptin, the top control of hypothalamic-pituitary-gonad (HPG) system, is the key neuropeptide involving in the ovulatory mechanism. The rise of *Kiss1* is shown prior to the ovulation in the mice [20]. Similarly, the elevation of plasma kisspeptin level is related with the preovulatory phase in the cows [21]. The feline kisspeptin is also shown to be located in both hypothalamic and extra-hypothalamic tissues [22,23]. The full-length cDNA of *Kiss1* has been isolated and cloned from the hypothalamic tissue of the domestic cat and caracal testis [23]. In the hypothalamus, kisspeptin neurons were identified in two distinct regions: preoptic area (Pea) and infundibular nucleus [23]. In the extra-hypothalamus, kisspeptin and its receptor were identified in the ovary and uterus [22]. Representatives of the different feline genera with different ovulatory patterns: almost exclusively induced (*Felis, Caracal and Panthera*) or spontaneous (*Neofelis*), shared an identical Kp10 sequence and an identical 3′ end of *Kiss1* [23]. Accordingly, the mechanism of kisspeptin neurons mediating GnRH release to induce ovulation in the musk shrew (*Suncus murinus*), a reflex ovulator, has been shown to be well conserved as in spontaneous ovulators [19]. Taken together, both ovulation induced by vaginal stimuli and spontaneous are likely controlled by the same kisspeptin mechanism. Thus, whether kisspeptin function is the real key factor regulating estrus patterns in the cats is yet to be elucidated.

Regardless of the ovulatory type, the corpus luteum is developed in the ovary after ovulation. The corpus luteum is a dynamic endocrine gland which is responsible for the production of progesterone. The rise of progesterone (>2 ng/mL) is measured in the peripheral blood at 1–2 days after the ovulation, and remains detectable for 36–38 days in non-pregnant and 64–67 days in pregnant domestic cats [24–26]. The non-ovulated luteal phase in the felids is usually half of the duration of pregnancy with the average 40.9 days (evaluated from the domestic cat, leopard cat, puma, ocelot, caracal, bay cat and Panthera lineages) [10].

2.2. Reproductive biology in the male

Ejaculate traits are documented for 28 of the 38 felid species [10]. Teratospermia (a semen with <60% morphologically normal sperm) is the common characteristics of poor semen quality presented in the felid species of small population which is usually associated with low genetic diversity. Moreover, captive environment such as single or paired living status can influence sperm quality. The example is shown in the clouded leopards, in which males housed with a female had a higher proportion of sperm with intact acrosome and lower proportion of sperm with bent midpiece and droplet than the males living singly [27]. However, the inability to uncoil the coiled sperm tail by demembranation, suggesting that the cause may be from testicular origin which is also a genetic related abnormality [28]. In cheetahs raised in public exposure in the North America, compromised motile sperm output unrelated to hyper-adrenal activity has been reported [29].

Seminal plasma of cats contains fluids secreted from bulbourethral and prostate glands. Recent study using ontology analysis has shown that 56%, 33% and 11% of proteins in the domestic cat seminal plasma functions as binding, catalytic and antioxidant activity, respectively, whereas 41% and 23% of sperm extracted proteins involve in catalytic activity and binding, respectively [30]. Moreover, 106 proteins are identified in the cat seminal plasma and 98 proteins are extracted from spermatozoa with 8 proteins in both seminal plasma and sperm extracts [30]. These indicate that epididymal and ejaculated spermatozoa are different in their biochemical properties. Thus, ejaculated sperm may not be substituted with epididymal sperm for all types of the studies. Apart from the different biological property of ejaculated and epididymal domestic cat sperm, the spermatozoa in different parts of the epididymis exhibit different susceptibility to osmotic stress with more tolerate in those from cauda than corpus and caput [31]. However, similar freezability is observed in the spermatozoa from the corpus and cauda epididiymal region [32]. Therefore, sperm collection from the corpus epididymis allows additional recovery of spermatozoa for preservation.

3. Gamete collection and cryopreservation

3.1. Ovarian stimulation

Ovarian stimulation in felids is commonly performed using equine chorionic gonadotropin (eCG), follicle stimulating hormone (FSH), gonadotropin releasing hormone (GnRH), GnRH analogues and opioid antagonists with varying results [8]. Porcine FSH (pFSH) is sometime chosen because it has amino acids homology with tiger FSH. Similarly, purified pFSH has a minimal LH activity resulting in a better quality of embryo production in the Siberian tigers [33]. However, FSH has short half-life, therefore, needs twice daily injections for 3–6 consecutive days to induce folliculogenesis. Apart from FSH, eCG is currently a hormone of choice in many felid species because it induces estrus in a single injection. However, the disadvantage of eCG is the production of anti-gonadotropin antibodies leading to decrease ovarian responsiveness if the hormone is administered repeatedly within 6 months [34]. The responsiveness of the ovary to exogenous gonadotropin is species-specific with the optimal eCG dosage being not associated with body weight [35]. Superovulation is commonly induced when 200 IU eCG was administered to the domestic cats, resulting in 39.1 oocyte ovulated per cat [36]. However, several animals did not respond to the eCG induction with unexplained causes [37]. Using *in vitro* culture, eCG stimulates follicular growth and gene expressions in the multilayered secondary follicles and antral follicles in the domestic cats have been reported [38]. Attempts to find a suitable exogenous hormone for ART in felids with less side effects are ongoing.

Developmental stages of ovarian follicles are divided into primordial, primary, secondary and antral follicles. In the ovaries of adult domestic cats, the average number of primordial follicles are estimated as 74,520 [39]. Using a cell dissection sieve, 90% of harvested follicles are primordial and primary follicles (diameter = 40–50 μm), and 10% are secondary follicles (<60 μm). Under the light and transmission electron microscopy, the cat ovaries present 37,853 ± 6118 preantral follicles, consisting of 87% primordial (22–41 μm), 10.4% primary (25–64 μm) and 2.3%

secondary follicles (43–110 μm) [40]. Only about 2% of all follicles leave the resting pool of primordial follicles and start growing [41].

3.2. Collection of oocytes and freezing

In vitro producing embryo from the oocytes has been developed in both domestic and wild cats in order to conserve the female gametes from the ovariohysterectomized or accidentally died animals. The cumulus oocyte complexes (COCs) are rescued from the ovaries and cultured. The good quality COCs (class I and II) have ability to develop more than the poor quality [42]. The *in vitro* maturation (IVM) is important process, allowing the good quality COCs to develop into metaphase II stage and prompt for fertilization [43]. After maturation, there are two techniques for *in vitro* insemination. First, *in vitro* fertilization (IVF) [44]. the viable spermatozoa penetrated to mature oocyte and fertilization occurred. Second, intracytoplasmic sperm injection (ICSI) [45], the ejaculated sperm [46], epididymal sperm [47] and testicular sperm [48,49] have been reported. The embryo from both IVF and ICSI have been transferred into egg donors, thereafter, into gonadotropin treated recipients [50]. The embryo transfer (ET) has been performed in both domestic [36,51] and wild cats using both fresh [36] and cryopreserved embryos [1]. Transferring embryos to either the oviduct or the uterine horn has resulted in pregnancies [52,53].

3.3. Culture of follicles and freezing

Gonadotropins and paracrine factors (the oocytes secrete paracrine factors such as growth differentiation factor 9 (GDF9) [54] and bone morphogenetic protein 15 (BMP15) [55] promote follicular growth and differentiation [56]. In addition, FSH plays an important role in promoting granulosa cell proliferation and differentiation from preantral follicles stage [57]. FSHR expression is triggered by oocyte-derived GDF9 [58]. FSH stimulation is also essential for LHCGR expression. LH also plays a major role in regulating follicle and oocyte maturation [59]. LH exerts its effects through binding to its receptor (LHCGR) and enhances final differentiation of the granulosa cells and the enzymes responsible for androgen production in the theca cells [60]. Besides the factors described above, various local factors such as insulin-like growth factor I (IGF-I) [61], epidermal growth factor (EGF) [62,63], fibroblast growth factor (FGF) [64] have to work synergistically in an autocrine or paracrine manner to enhance granulosa cell proliferation, maintenance of follicular integrity, and the survival of oocytes *in vitro*. In the cats, primordial and primary follicles within ovarian tissues sustain their viability up to 14 d after cultured on an agarose gel which allows the tissue to expose to the oxygen in the *in vitro* environment [65]. Culture of cat preantral follicles with activin A promotes growth and viability after 14-day [66]. The successful experiment is carried out in the mouse, resulting in normal offspring from preantral follicles culture [67]. Although the results from the use of two-dimensional culture system are successful in the rodent model, it does not support follicular development in the larger mammals [68]. After three to five days of culture, the follicles lose their structure by attachment on the culture plate surface [69]. This results in follicle extrusion, basement membrane disruption and dysfunction of the gap junctions, which play a key role on the communication between the oocyte and cumulus cells [70]. Dysfunction of the gap junctions can cause arrest in follicular growth and impaired meiotic competence [71]. Thus, the three-dimensional culture system (alginate hydrogel) imitates ovarian stromal structure has been developed to sustain follicular architecture. To cryopreserve cat ovarian tissues, sucrose supplementation is suggested to protect follicular viability, follicle morphology, gap junction protein, and stromal cell apoptosis [72]. In addition, viability and growth of preantral follicles derived from cryopreserved ovarian tissues of a cheetah (*Acinonyx jubatus*) post-mortem have been reported [73].

3.4. Collection of semen and freezing

Semen in the wild cats are obtained by anesthesia and electroejaculation. Alternatively, with medetomidine administration, semen can be forced from the constricted ejaculatory ducts into the urethra through a catheter [74]. This method is so-called urethral catheterization. It has been applied to the Asian lions, Asian golden cats (*Catopuma temminckii*) [75] and jaguars [76]. Recently, a novel combination of urethral catheterization for domestic cat sperm recovery and vitrification offers advantages of using the simple and minimal equipment [77]. In the wild cats, spermatozoa are obtained post-mortem from caudal epididymis by tissue chopping into small pieces up to 24 h after death [78]. The domestic cat sperm obtained by epididymal slicing have equal quality as that collected by electroejaculation [79]. Apart from the electroejaculation and epididymal retrievals, live cat sperm can be recovered from testes refrigerated for seven days [80]. Cryopreservation of testicular spermatozoa as a minced suspension provided higher sperm with intact plasma membrane than that in the testicular fragment form [81].

4. Artificial insemination

Artificial insemination is commonly applied to propagate populations of domestic animals with highly producing traits and the endangered species with valuable genetic. Germplasm banks including frozen semen of valuable males are closely associated with the improvement of breeding programs by overcoming behavior incompatibility between individuals and enhancing founder representation in small population [82]. Moreover, it helps natural mating, particularly for animals that are separately housed in distance location [83]. The success of AI in felids is related to several factors of female, male and AI technique. The factors include: 1) ovarian responsiveness to stimulation, 2) precise time of insemination 3) sperm quality (types of sperm storage prior to insemination and number of sperm to be inseminated, and 4) site of insemination. Ovarian response to the exogenous gonadotropins is the major key for achieving pregnancy. Several ovarian stimulation protocols for AI have been reviewed in nine wild felids [84]. The protocol that provides similar response to natural cycle is considered the best, otherwise it increases the incidences of ovarian hyperstimulation, premature luteolysis and impaired embryo development and gestation [12,84–86]. Moreover, species with spontaneous ovulation are a challenge for developing successful ARTs, because the sporadic production of CL and progesterone can compromise effectiveness of gonadotropins, altering the endocrine environment and causing failure of fertilization and embryo development [87]. Accordingly, the pregnancy from AI can be enhanced by the first reset all ovarian activity before gonadotropin administer is a high priority [35,87]. Recently, ovulation induction after natural cycle using 20 μg GnRH analogue buserelin-acetate (Receptal®) on Day 5 from estrus onset, about 24 h before AI has been reported in lion and resulted in 4/14 pregnancy followed by the delivery of two cubs from one female [88]. Timing the AI also influences AI success. Successful of insemination during both pre- and post-ovulations has been reported in domestic cat [89,90]. However, in cheetah, AI before ovulation resulted in decreasing of pregnancy incidence, which may cause using anesthesia prior to ovulation compromised ovulation [35,91].

According to the site of insemination, AI is divided into three methods; 1) intravaginal, 2) intrauterine and 3) intratubal

inseminations. Both surgical and non-surgical (using AI catheter or laparoscope) approaches have been used to access the insemination site. The application of AI has been reported in 11 wild felid species with variable success (Table 1). It has been demonstrated that fresh semen is required for vaginal AI as showed the higher conception success when inseminate with higher number of sperm (50% with 10–50 $\times$ 10^6 sperm and 78% with 80 $\times$ 10^6 sperm, respectively). Moreover, deeper sperm deposition sites such as intrauterine and intratubal insemination, required lower number of sperm cells with acceptable conception rate. The conception rate was 50 and 80% following deposition of 6.2 $\times$ 10^6 and 8 $\times$ 10^6 sperm at intrauterine site whereas deposited 4 $\times$ 10^6 spermatozoa at intratubal site obtained 43% conception rate [109]. While the frozen-thawed sperm are used, only intrauterine insemination is recommended [5]. In wild felids the teratospermia likely occurs in several species, in particular in the population lacking genetic variability [82]. The primary limitation of intrauterine AI is that the technique requires high number of motile sperm cells to ensure sperm penetration through the utero-tubal junction into the oviducts to achieve fertilization. In 1992, laparoscopic intrauterine (LU) has been used to achieve pregnancy in the leopard cats [95], thereafter it has been applied to other wild felids. Since 2001, intratubal or oviductal AI has been developed to improve pregnancy outcomes by allowing deposition of semen in closer proximity to the site of fertilization [109]. Furthermore, small number of frozen-thawed sperm (10 $\times$ 10^6 sperm) collected from caudal epididymis is capable of achieving pregnancy in the domestic cats [110]. Thereafter, in 2012 laparoscopic oviductal (LO) AI has been introduced to minimize surgical wound, leading to improved conception rate [98]. The LO AI is used successfully in the domestic cats and subsequently applied to several wild felids i.e. Pallas' cats (Otocolobus manul) [98], ocelots (Leopardus pardalis) [98], Amur tigers (Panthera tigris ssp. *altaica*) [108] and clouded leopards [101]. To highlight the effort of clouded leopard breeding consortium, the collaboration between two continents in United State and Thailand provide better understanding of reproductive biology, behavior and nutrition in this species. Moreover, live cubs born have been demonstrated using LU AI with fresh sperm (89 $\times$ 10^6) at 45 h post-hCG [100], LO AI with chilled motile sperm (10.7 $\times$ 10^6) at 45 h post-pLH [101] and LO AI with 30 $\times$ 10^6 motile frozen-thawed sperm at 44 h post-pLH [102] were reported. As the progress of producing wild felid offspring by AI, it is promising for sustaining their genetic diversity in the future.

5. *In vitro* fertilization and intracytoplasmic sperm injection

In the past decade, many attempts have been made to improve the efficiency of cat embryo production. However, producing cat embryos is restricted by their nature, unclear fundamental physiology and appropriate *in vitro* conditions. The *in vitro* maturation rate of cat oocytes varies between 40 and 60% [111], and depends on many factors i.e. the composition of maturation medium, the stage of the reproductive cycle and supplementation in the medium [112–121]. Furthermore, two stages of embryo developmental arrest have been observed in cat embryos cultured *in vitro*, at 5–8 cell stage, which correspond to the transition period from maternal to embryonic control, and are at morula to blastocyst stage [122]. The first birth of domestic cat kittens by IVF has been reported since 1988, resulting in the improvement of fundamental embryology and the embryo production techniques. Moreover, the achievement in domestic cat aroused the development of ART in wild cat for conservation. Thereafter, successful wild cat embryo production by IVF has been reported in 16 wild cat species i.e. leopard cat (Prionailurus bengalensis) [123], puma (Puma concolor) [124], cheetah [125], jungle cat (Felis chaus) [126], jaguar [127], tigrina (Leopadus tigrinus) [128], clouded leopard [129], tiger [33,130], fishing cat (Prionailurus viverrinus) [126], Indian desert cat (Felis silvestris *ornate*) [126], African wildcat (Felis silvestris *lybica*) [131], caracal (Caracal caracal) [53], serval (*Leptailurus serval*) [53], black-footed cat (Felis nigripes) [132], sand cat (Felis margarita) [133] and

Table 1
Estrus and ovulation inductions, sperm types and site of AI on pregnancy success in domestic and wild felids.

Body size	Species	Estrus/ovulation induction	Sperm type	AI mean/sperm deposition	Pregnancy success (%)	References
Small (<6.5 kg)	Domestic cat	natural estrus/hCG	Fresh	Vagina	7/9 (77.81)	[92]
	Domestic cat	natural estrus/pFSH/hCG	Frozen	Vagina	6/56 (10.7)	[93]
	Domestic cat	natural estrus/hCG	Fresh	Laparotomy intrauterine	8/10 (80)	[90]
	Domestic cat	natural estrus/hCG	Frozen	Laparotomy intrauterine	8/14 (57.1)	[94]
	Domestic cat	natural estrus/hCG	Frozen	Transcervical	5/12 (41.7)	[5]
	Domestic cat	eCG/pLH	Fresh	LO	3/3 (100)	[12]
	Leopard cat	eCG/hCG	Fresh/Frozen	LU	2/2 (100)	[95]
	Amur leopard cat	eCG/hCG	Fresh	Laparotomy intrauterine	1/2 (50)	[96]
	Tigrina	eCG/hCG	Fresh	LU	1/4 (25)	[97]
	Pallas's cat	eCG/pLH	Fresh	LO	1 pregnant	[98]
Medium (7–30 kg)	Ocelot	eCG/hCG	Fresh	LU	1/4 (25)	[99]
	Ocelot	eCG/hCG	Frozen	LU	1/4 (25)	[97]
	Ocelot	eCG/pLH	Fresh	LO	1 pregnant	[98]
	Clouded leopard	eCG/hCG	Fresh	LU	1/20 (5)	[35,100]
	Clouded leopard	eCG/pLH	Fresh	LO	1/4 (25)	[101]
	Clouded leopard	eCG/pLH	Frozen	LO	1 pregnant	[102]
	Snow leopard	eCG/hCG	Fresh	LU	1/15 (6.7)	[86]
Large (35–135 kg)	Leopard	natural estrus/hCG	Fresh	Transcervical	1/1 (100)	[103]
	Cheetah	pFSH/hCG	Fresh/Frozen	Transcervical	0/23 (0)	[104]
	Cheetah	eCG/hCG	Fresh	LU	6/13 (46.2)	[35]
	Cheetah	eCG/hCG	Frozen	LU	3/11 (27.2)	[35,105]
	Puma	eCG/hCG	Fresh	LU	1/8 (12.5)	[106]
	Tiger	eCG/hCG	Fresh	LU	1/10 (10)	[11,107]
	Amur tiger	eCG/pLH	Fresh	LO	1 pregnant	[108]
	Lion	natural estrus/GnRH	Fresh	Vaginal/Transcervical	4/14 (28.6)	[88]

Abbreviation: LU, laparoscopic uterine; LO, laparoscopic oviductal AI.

ocelot [133]. The live born by IVF has been reported in the last nine species. However, there has not been reported about live wild cat born by IVF since 2012.

Ten year after the first IVF kittens born, ICSI has been applied successfully in domestic cat [134]. By the same technique, domestic cat cryopreserved testicular sperm has been successfully used to produced embryos, followed by the live kittens born after transferred the frozen embryos [135]. The ICSI has been efficiently applied in jaguarondi (using cryopreserved ejaculated sperm) [134], lion (using cryopreserved epididymal sperm) [136] and fishing cat (using cryopreserved ejaculated sperm) [53] and yielded 56–70% cleavage rate, however, pregnancy could not be established. Furthermore, it has been shown that 34–39% of lion oocytes retrieved at eight h post-mortem could reached MII stage, at 32–34 h after IVM and ICSI provided the opportunity to produce embryos at 31–58% cleavage rate, and 29–50% of cleave embryos were subsequently developed to blastocyst [137]. Since wild cat oocytes are not available, interspecific ICSI has been used to investigate fertilizing ability of various animals including jaguar freeze-dried sperm in mouse oocytes and it was found that 96% of successful injected oocytes formed pronuclei [138]. Moreover, the blastocysts could be generated without or with ionomycin activation after injected cheetah and leopard spermatozoa to domestic cat oocytes (32.6 V S. 21% and 9.8 V S. 21%, respectively) [139].

In overview, producing wild cat embryos by and IVF and ICSI has not been in progress recently, particularly in term of production of live born, which may be because of unavailable donor oocytes from wild cats, inaccurate ovarian response after stimulation, small number of recipients, complex and expensive technique (for ICSI) and skillful technician required (for ICSI). Thus, the studies related to IVF/ICSI augment are required for further application of the technologies in wild cat conservation.

6. Somatic cell nuclear transfer

Somatic cell nuclear transfer in felid species has been developed sine year 2002 in domestic cats [120,140] and has been used as a model for nondomestic cats. Many studies have been conducted to improve the poor development of cloned domestic cat embryos cultured *in vitro* [116,141,142]. The SCNT is considered as a potential tool for the molecular tracking of nucleo-cytoplasmic interaction, the transmission of two cytoplasmic populations that will influence embryonic development and survival after implantation and producing transgenic cats, which are useful models for the study of hereditary diseases in human [143]. Besides that, it also provides an opportunity for producing offspring of endangered felids. Producing the embryos from cryobank-derived cells collected from alive or dead animals by SCNT is only a way to restore or conserve rare species. In endangered felids, due to a lack of oocytes, inter-species/generic SCNT or the process of transferring the donor cells from one species/genus into the oocytes of another has been performed to produce their embryos. The host oocytes from closely related and unrelated animals have been used as alternatives. Of eight felid lineages, reconstructions of domestic cat and wild cat donor cells with domestic cat recipient cytoplasm have been reported in six lineages; domestic cat, leopard cat, bay cat, caracal, panther and puma lineages with total of 11 species and a hybrid Bengal cat. Several studies have demonstrated the potential of rabbit and bovine recipient cytoplasm for reprogramming of domestic cat and four wild cat species (Table 2). The success of live offspring production in wild cat has been shown in only three species of two lineages (domestic cat and caracal lineages), the African wildcat [141] and sand cat [156] born by inter-species SCNT and caracal born by intra-species SCNT [148]. Although the successful generation of cloned animals and a better understanding of SCNT have been demonstrated, the efficiency of SCNT offspring production is very limited in felid species being about 1% (kitten/total embryo transfer). Besides, it is still impaired by the main factors i.e. the biological complexity of nuclear reprogramming and the availability of host oocyte donor and/or suitable recipients.

Other inter-generic SCNT embryo production has been demonstrated in the tiger [149], rusty spotted cat (Prionailurus rubiginosus) [157], leopard cat [144,146], marbled cat (Pardofelis marmorata) [153], black-footed cat [143], flat-headed cat (Prionailurus planiceps) [147], tiger [145], cheetah [150] and Bengal cat [145] using domestic cat oocytes as the recipient cytoplasm. Surprisingly, the domestic cat enucleated oocytes had high capacity to reprogram the wild cat donor cells to cleavage embryos. However, the cleavage embryos reached poor blastocyst success (2.6–26.7% in domestic cat lineage, 37.2% in hybrid domestic cat and leopard cat lineages, 8.6–26.2% in leopard cat linage, 5.4% in bay cat linage, 3.4–9% in panther lineage and 0–19.1% in puma linage). The results indicate that phylogenetic distances between donor cell and recipient cytoplasm seem to be involved with the embryo developmental success to the blastocyst. It has been reported that major constraints to the development of inter-generic NT embryos likely include inadequate nuclear reprogramming [141] and chromosomal abnormalities derived from karyotypically abnormal somatic cells [158]. Furthermore, other external factors such as methylation [159] and co-existed mitochondrial DNA (mtDNA) [155] have been involved. In overall, there has not been much progress of SCNT since there are several limitations including great skill for researchers, expensive laboratory facilities, scientific equipment and supplies, animal colonies and research funding. Only recent studies in 2015 demonstrated the improvement of SCNT efficiency by zona-free cloning technique combined with embryo aggregation, which improved about 10% of blastocyst success, as shown in domestic cat, Bengal cat, tiger and cheetah [145,150]. This achievement encourages the positive challenge for making SCNT applicable for endangered felid embryo production, which will be useful for further cellular and molecular investigations.

7. Pluripotent stem cell

Cell technologies in felid species have been performed in the domestic cats, e.g. pluripotent stem cells (PSC), fibroblasts [160–165], mesenchymal stem cells (MSC) [166–168], peripheral blood mononuclear cell (PBMC) [169], cancer cell lines (feline mammary cancer and feline histolytic sarcoma cell line) [170,171] and testicular tissue fragments [172]. These established cells are generated to resolve key issues in clinical veterinary medicine and served as *in vitro* culture model, as summarized in Fig. 2 and Table 3. The pluripotent stem cells of the domestic cats have been established from two sources: embryonic stem cells (ESCs) derived from blastocysts [161,163,173,174] and induced pluripotent stem cells (IPSCs) generated from cellular reprogramming of the fibroblasts [160,176]. To derive feline ESCs, blastocysts are obtained via *in vivo* technique (ovariohysterectomy) and IVF. The inner cell mass (ICM) of the blastocyst, the major source of PSC, can be harvested by immunosurgery, mechanical isolation or ICM outgrowth. Early establishment of cat ESCs faced difficulty due to spontaneous differentiation. The immunosurgery has shown to maintain better undifferentiated morphology than the mechanical isolation resulting in higher ESC yields [161]. Moreover, the cat ESCs from IVF-derived blastocysts maintained in undifferentiated state longer than those from *in vivo*-derived blastocysts [161,173]. Therefore, to maintain sufficient undifferentiation and pluripotency, cat ESCs are cultured with human basic fibroblast growth factor (bFGF) and murine leukemia inhibitory factor (LIF) [161]. In addition to feline LIF, cat ESCs can be maintained under the treatment of inhibitors,

Table 2
In vitro development of intra-species, inter-species and inter-generic cloned felis embryos reconstructed from enucleated domestic cat, rabbit and bovine oocytes and progress after ET.

Donor cell lineage	Species of donor cell	Species of enucleated oocyte	Cleavage/total fused (%)	Blastocyst/total cleaved (%)	Pregnancy success (%)	Kitten/total embryos transferred (%)	Reference
Domestic cat	Domestic cat	Domestic cat	201/284 (70.8)	32/201 (15.9)	4/18 (22.2)	6/812 (0.7)	[144]
Domestic cat	Domestic cat	Domestic cat	99/113 (87.6)	31/99 (31.3)	–	–	[145]
Domestic cat	Domestic cat	Domestic cat	107/109 (98.2)[a]	52/107 (48.6)[a]	–	–	[144]
Domestic cat	African wild cat	Domestic cat	1017/1282 (79.3)	272/1017 (26.7)	17/40 (42.5)	17/1627 (1.0)	[143]
Domestic cat	Sand cat	Domestic cat	499/558 (89.4)	83/499 (16.6)	14/45 (31.1)	14/1600 (0.9)	[143]
Domestic cat	Black-footed cat	Domestic cat	428/502 (85.2)	11/428 (2.6)	9/20 (45.0)	0	[143]
Domestic cat-Leopard cat	Bengal cat	Domestic cat	129/154 (83.8)	48/129 (37.2)	–	–	[145]
Domestic cat-Leopard cat	Bengal cat	Domestic cat	98/105 (93.3)[a]	46/98 (46.9)[a]	–	–	[145]
Leopard cat	Rusty spotted cat	Domestic cat	65/76 (85.5)	17/65 (26.2)	0/5 (0)	0	[143]
Leopard cat	Leopard cat	Domestic cat	45/54 (83.3)	3/45 (6.7)	–	–	[146]
Leopard cat	Leopard cat	Domestic cat	147/218 (67.4)	16/147 (57.1)	4/7 (57.1)	0	[144]
Leopard cat	Flat-headed cat	Domestic cat	58/60 (96.7)	5/58 (8.6)	0/9 (0)	0	[147]
Bay cat	Marbled cat	Domestic cat	56/60 (93.3)	3/56 (5.4)	–	–	[144]
Caracal	Caracal	Domestic cat	N/A	N/A	N/A	1 kitten	[148]
Panthera	Tiger	Domestic cat	N/A (65)	N/A (9)	–	–	[149]
Panthera	Tiger	Domestic cat	59/63 (93.7)	2/59 (3.4)	–	–	[145]
Panthera	Tiger	Domestic cat	63/66 (95.9)[a]	8/63 (12.7)[a]	–	-	[145]
Puma	Cheetah	Domestic cat	89/102 (87.2)	17/89 (19.1)	–	–	[150]
Puma	Cheetah	Domestic cat	88/91 (96.7)[a]	26/88 (29.5)[a]	–	–	[150]
Puma	Asiatic cheetah	Domestic cat	156/483 (32.3)	0	–	–	[151]
Domestic cat	Domestic cat	Rabbit	127/189 (67.2)	13/127 (10.2)	–	–	[152]
	Domestic cat	Rabbit	22/26 (85)	2/22 (9.1)	–	–	[153]
Bay cat	Marbled cat	Rabbit	26/26 (100)	3/26 (11.5)	–	–	[153]
Panthera	Leopard	Rabbit	216/320 (67.5)	13/216 (6.0)	–	–	[154]
Panthera	Tiger	Rabbit	214/310 (69.0)	14/214 (6.5)	–	–	[154]
Panthera	Lion	Rabbit	197/268 (73.5)	13/197 (6.6)	–	–	[154]
Domestic cat	Domestic cat	Bovine	175/202 (86.6)	0	–	–	[155]

[a] Embryo produced by embryo aggregation method.

e.g. GSK3 inhibitor (CHIR99021) and/or MEK inhibitor (PD0325901), [175]. This combination of LIF and inhibitors is known as "2i/LIF". Despite various methods have been utilized for the derivation of cat ESCs, modes of pluripotency maintenance are different from the mouse model [175]. All ESCs exhibited key characteristics of pluripotent stem cells including positive alkaline phosphatase activity [161,163,173], upregulation of core pluripotency genes similar to the blastocysts – OCT4 (POU5F1) [161,163,173,174], SOX2 [161] and NANOG [161,174], and presence of cell surface markers for pluripotent stem cells-SSEA1 [161,163,173], SSEA3 [173]and SSEA4 [163,173]. Interestingly, cat ESCs exhibited changes in stem cell signaling pathway including upregulation of STAT3 and downregulation of LIF-receptor compared to cat ICM under 2i/LIF condition.

Recently, feline IPSC derived from the domestic cat has been successfully established [160,176]. These studies highlight key requirements in cat pluripotency induction and maintenance, supporting previous cat ESC data, in particular different types of medium and cytokine combination: requirement of feline LIF under medium with serum [160] and feline LIF and 2i (together with human LIF) under serum-free medium with proprietary compounds (StemFlex) [176]. Human reprogramming factors including OCT4, SOX2, C-MYC, KLF4 and NANOG in both studies were sufficient to induce pluripotency from the adipose tissue derived fibroblasts and fetal tissue. This also suggests that using the five factor-reprogramming cocktail is sufficient to reprogram cat somatic cells in different ages [160,176]. Introduction of these exogenous factors was carried out by retrovirus and lentivirus treatment to induce pluripotency [160,176]. Hence, the introduced genes expressing reprogramming factors from retrovirus and lentivirus were still present in the feline IPSC lines although transgene expression can be silenced after doxycycline-removal [176] This feline IPSC lines exhibited pluripotency properties such as presence of core pluripotency markers and ability to form embryoid body. Nevertheless, some differentiation genes upregulating feline IPSC compared to the cat fibroblast, suggested that the mixture of fully reprogrammed cells and partially reprogramed cells with spontaneous differentiation was necessary [176]. In wild felids, IPSCs are only established *in vitro*. IPSCs provide alternative source of pluripotent stem cells instead of ESCs which are difficult to obtain due to unavailability of oocytes and embryos from wild felids. Wild cat IPSCs were generated from the adult fibroblasts of four wild felid species: African serval (*Leptailurus serval*, least concern), Asian bengal tiger (*Panthera tigris*, endangered status), snow leopard (*Panthera uncia*, vulnerable) and South American jaguar (*Panthera onca*, nearly threatened) [164,165] Retrovirus-based reprogramming introducing human *OCT4*, *SOX2*, *C-MYC*, *KLF4* and *NANOG* cDNA has been carried out to establish these wild IPS cells with certain pluripotent characteristics. In the absence of NANOG, the wild cat IPSCs maintained its undifferentiation state less than seven passages [164].This emphasizes the important role of NANOG in establishing pluripotency of the domestic and wild cats. In addition, murine LIF was sufficient for stable IPS clones. Hence, both domestic cat and wild cat pluripotent stem cells require at least a cell signaling mechanism called as "LIF-Janus kinase (JAK)-Signal transducer and activator of transcription (STAT), LIF/JAK/STAT"

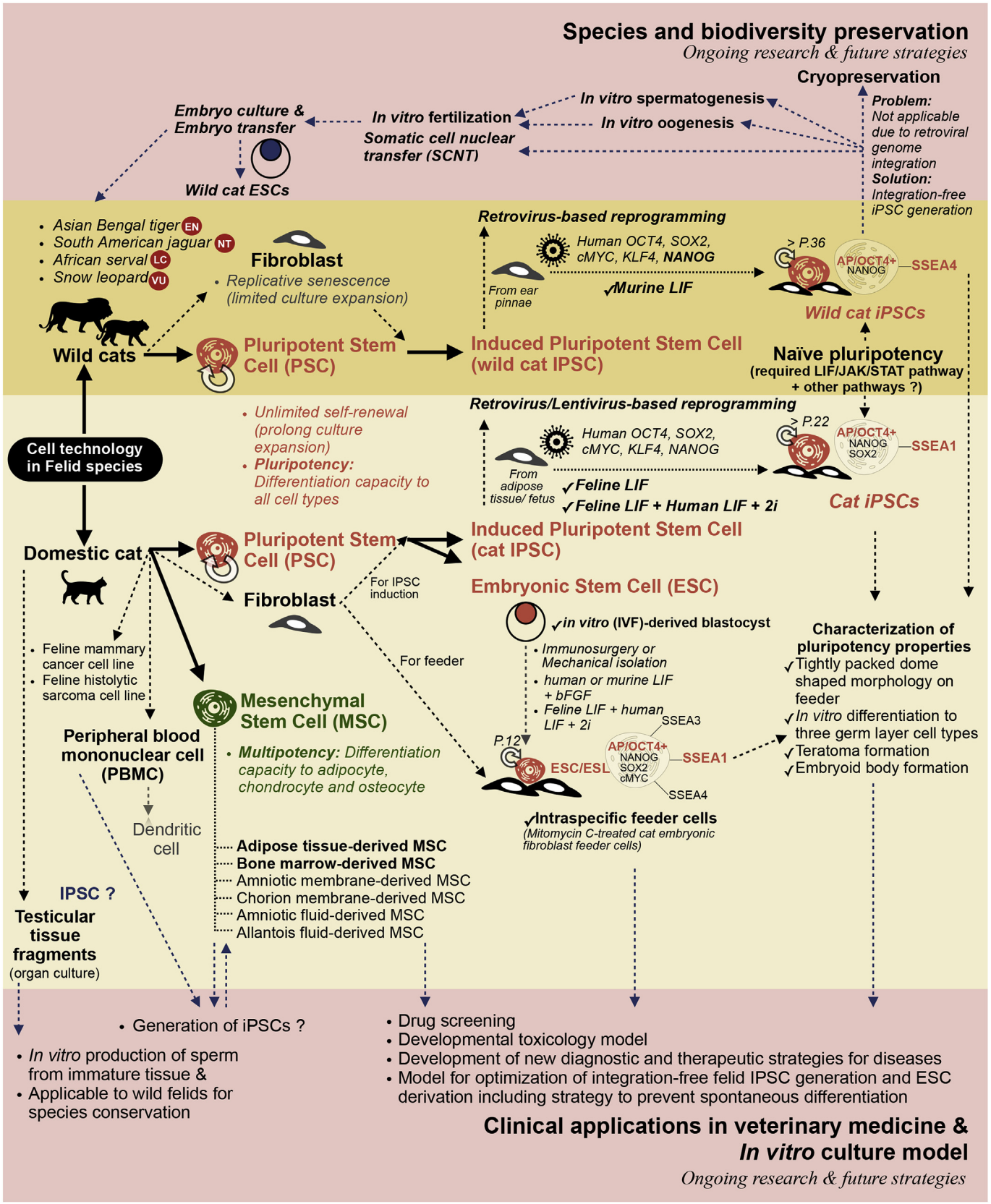

Fig. 2. Cell technology to derive felid pluripotent stem cells including embryonic stem cells (ESC) and pluripotent stem cells (IPSC) and other cell types. Pluripotent stem cells and other cell types such as mesenchymal stem cells (MSC), peripheral blood mononuclear cell (PBMC) and spermatogonium culture from testicular fragment were established in domestic cat. These cells provide potential applications in veterinary medicine. The generation of pluripotent stem cells in wild felids were carried out only for IPS cells, which can provide the benefit for future conservation research. Abbreviation: EN, endangered status; NT, near threatened status; LC, least concern; VU, vulnerable; 2i, 2 inhibitors including GSK3β inhibitor and MEK inhibitor; P., passage; AP, alkaline phosphatase.

Table 3
Pluripotent stem cells and fibroblasts for IPSC generation in domestic cat and wild felid species.

Type of cell	Cells	Sources	Unique culture conditions	Characterizations	References
Domestic cat					
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vitro*-derived blastocysts and *In vivo*-derived blastocysts	Standard mouse ES cell medium on inactivated mouse embryonic fibroblasts	After 3 weeks, ESCs from *in vitro*-derived blastocyst and *in vivo*-derived blastocyst differentiated regardless addition of murine or human LIF	[162]
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vitro*-produced blastocysts	Medium containing FBS and KSR with addition of 40 ng/mL murine LIF, 5 ng/mL human bFGF	Positive AP staining and expression of pluripotent markers including OCT4, SSEA1, SSEA4	[163]
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vivo*-derived blastocysts ICM isolation: mechanical segregation	Examination of medium with different serums: KSR or FBS, supplemented with 1000 IU/ml Murine LIF and cESCs were cultured on MCC-CEF	Positive AP staining of cESCs in both KSR and FBS medium and expression of pluripotency markers including OCT4, SSEA1, SSEA3, SSEA4. Mechanical passaged cESCs in FBS medium (P.6) can be maintained longer in culture than in KSR medium (P.4) while enzymatic passaged cells differentiated early (P.1–2). cESCs exhibited capacity to *in vitro* differentiation to embryoid body.	[173]
Fibroblast for feeder	Cat Embryonic Fibroblast (CEF) for feeder	Fetus at 4 weeks of gestation	Standard fibroblast culture medium and Mitomycin C treatment (10 μg/mL) for 2.5 h	Not examined/used for feeder to support IPSC generation	[173]
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vivo*-derived blastocysts	Medium containing FBS and KSR with 1000 IU/ml Murine LIF and cESCs were cultured onMCC-CEF	Expression of core pluripotency markers including *OCT4* (*cPOU5F1*) and *NANOG* (*cNANOG*)	[174]
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vitro*-produced blastocysts	Medium containing FBS and KSR with addition of 40 ng/mL murine LIF, 5 ng/mL human bFGF. cESCs were cultured on MCC-MEF or MCC-CEF	Positive AP staining and expression of pluripotency markers including *OCT4*, *NANOG*, *C-MYC*, *SOX2* *SSEA1* (negative for SSEA4). cESCs derived from immunosurgery (65–102 days) can be maintained longer than those from mechanical isolation (15–60 days). Culture of cESCs on CEF supported its propagation better than those seeded on MEF.	[161]
Fibroblast for feeder	Cat embryonic fibroblast (CEF) for feeder (MCC-CEF)	Fetal visceral tissues from fetus at day 35 of gestation	Standard fibroblast culture medium and Required higher dose of mitomycin C at 30–40 μg/mL for 5 h (10 μg/mL for 2.5 h for MEF)	Not examined/used for feeder to support IPSC generation	[161]
Pluripotent Stem Cell: Embryonic Stem Cell	Cat embryonic stem cell (cESC)	*In vitro*-produced blastocysts ICM outgrowth	KDMEM and NBM with 5% KSR, 2% B27 and 1% N2; 10 ng/mL feline LIF, 10 ng/mL human LIF, 3 μM GSK3 inhibitor, 1 μM MEK inhibitor, 10 μM ROCK inhibitors on MCC-MEF	RT-PCR showed similar pluripotency gene expression to ICM, except upregulation of STAT3 and downregulation of KLF4, CMYC and LIF receptor; IF showed lower level of NANOG, POU5F1 and SOX2. cESCs can be maintained in culture more than 10 passages.	[175]
Pluripotent Stem Cell: Induced Pluripotent Stem Cell	Feline induced pluripotent stem cell	Feline fibroblasts from adipose tissue infected with retrovirus carrying human *OCT4, SOX2, C-MYC, KLF4, NANOG*	Medium containing FBS with 10 ng/mL feline LIF and cultured on MCC-MEF	IPS colony appeared with 3–5 days post infection. IPSCs exhibit positive AP and pluripotency markers including OCT4, SOX2, NANOG, SSEA1 except SSEA4. IPSCs can be maintained in undifferentiated state more than 22 passages. IPSCs can also differentiate into ectoderm, mesoderm and endoderm.	[160]
Fibroblast for IPSC generation	Feline adipose tissue-derived fibroblasts	Adipose tissue	Standard fibroblast culture medium with 10 ng/mL bFGF	Not examined/used for IPSC generation	[160]
Pluripotent Stem Cell: Induced Pluripotent Stem Cell	Feline induced pluripotent stem cell	Cat fetal fibroblasts (CFF) Infected with lentivirus carrying *human OCT4, SOX2, C-MYC, KLF4, NANOG* under tet-on promoter (Doxycycline inducible)	Induced cells were cultured in medium containing FBS and doxycycline and then changed to commercial StemFlex™ medium supplemented with 10 ng/mL feline LIF, 10 ng/mL human LIF, 3 μM GSK3 inhibitor, 1 μM MEK inhibitor, 10 μM ROCK inhibitors and doxycycline, on MCC-MEF.	IPS colony emerged at day 14 after Doxycycline induction; IPSCs exhibited positive signal for AP and pluripotency markers (Upregulation of OCT4 and SOX2; downregulation of CMYC). IPSCs can be maintained more than 10 passages without transgene. IPSCs can differentiate to embryoid body with the presence of three-germ-layer cell types.	[176]
Fibroblast for IPSC generation	Cat fetal fibroblasts (CFF)	Second-trimester fetus	Standard fibroblast medium with FBS	Not examined/used for IPSC generation	[160]
Wild cats					
	Snow leopard IPSC		Medium containing FBS and murine LIF, on MCC-MEF		[164]

Pluripotent Stem Cell: Induced Pluripotent Stem Cell		Ear pinnae-derived adult fibroblast infected with retrovirus carrying human *OCT4*, *SOX2*, *C-MYC*, *KLF4* and/or *NANOG* (OSMKN or OSMK)			IPS colony appear on day 3 post infection. OSMKN IPSCs (>P.36) can be maintained longer than OSMK IPSCs (P.4). OSMKN IPSCs expressed AP, OCT4, NANOG, SSEA4. Also, IPSCs can form teratoma with three-germ-layer cells
Fibroblast for IPSC generation	Snow leopard adult dermal fibroblast	Ear pinnae tissue	Standard fibroblast medium with FBS		Not examined/used for IPSC generation
Pluripotent Stem Cell: Induced Pluripotent Stem Cell	Bengal tiger IPSC Jaguar IPSC Serval IPSC	Ear pinnae-derived adult fibroblast infected with retrovirus carrying human *OCT4*, *SOX2*, *C-MYC*, *KLF4* and/or *NANOG* (OSMKN or OSMK)	Medium containing FBS and murine LIF, on MCC-MEF	[165]	IPS colony appear on day 3 post infection. OSMKN IPSCs (>P.14) can be maintained longer than OSMK IPSCs (P.7). IPSCs expressed AP, OCT4, NANOG, SSEA4. Also, IPSCs can form embryoid body and teratoma with three-germ-layer cells
Fibroblast for IPSC generation	Bengal tiger, jaguar, and serval fibroblast	Ear pinnae tissue	Standard fibroblast medium with FBS	[165]	Not examined/used for IPSC generation

Abbreviation: AP, Alkaline phosphatase; bFGF, Basic fibroblast growth factor; FBS, Fetal bovine serum; hrs, hours; K-DMEM, Knockout™ DMEM; KSR, Knockout serum replacement; LIF, Leukemia inhibitory factor; MCC-CEF, Mitomycin C-treated cat embryonic fibroblast; MCC-MEF, Mitomycin C-treated mouse embryonic fibroblast; NBM, Neural Basal Medium; P., Passage.

pathway to support naïve pluripotency. However, the wild cat IPSCs from these studies cannot be used for species preservation due to the presence of unremovable exogenous genes and retroviral DNA in host genome. It is still challenging to establish IPSCs without integration of reprogramming factor DNA into the host genome (integration-free IPSC) from available reprogramming methods e.g. episomal vector, sendai virus, mRNA transduction and self-replicating RNA. Future successful generation of integration-free wild cat IPSC will provide tremendous source for cell differentiation into all cell types in particular gametes (*in vitro* spermatogenesis and *in vitro* oogenesis) and even a whole animal body via SCNT.

8. Conclusion

Reproductive knowledge in felids have been continually studied from basic biology to advance biotechnologies. The zoos play the important role in felid conservation by establishing assisted reproductive biotechnology protocols to create *ex situ* breeding programs, provide knowledge as well as maintain gamete and cell banking. Successes of cryo-banking would sustain genetic diversity to finally sustain the numbers of endangered felid species.

CRediT authorship contribution statement

Ampika Thongphakdee: Conceptualization, Data curation, Writing - original draft, Visualization. **Woranop Sukparangsi:** Data curation, Writing - original draft. **Pierre Comizzoli:** Writing - review & editing. **Kaywalee Chatdarong:** Writing - original draft, Supervision.

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