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# When to cryopreserve and when to let it go? A systematic review of priorities in wild animal cryobanking

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ABSTRACT

With increasing numbers of species threatened with extinction, collecting and conserving living samples is important for the long-term conservation of animal populations. Globally, many cryobanks have been developed to preserve animal tissues for future use in wildlife conservation. However, to date, there has been no attempt to review the purpose, priorities and direction of these cryobanks. A systematic review was undertaken using Web of Science, Scopus, and Google Scholar to determine the most common priorities identified in the cryobanking literature. The types of species being recommended for cryobanking, cell types, and recommended numbers of samples and number of individuals were also recorded for cryobanking efforts. Overall, 13,287 papers were identified, of which 794 were selected for full-text review. For wildlife, the most frequently cited priority was to select based on threat level, with convenience sampling and genetic diversity featuring as the second and third most common priorities. In terms of cell type, sperm featured most frequently in cryobanking literature, potentially due to its ease of use in animal breeding programmes. Somatic cells and stem cells featured more commonly in more recently published literature. Looking ahead, cryobanks should consider their priorities and records to ensure they are collecting samples with a meaningful use for future conservation efforts. Greater collaboration between cryobanks can aid in important sample acquisition and storage and in sharing cryopreservation priorities.

## 1. Introduction

Projections indicate that the global human population may exceed 10 billion by the year 2100 [1]. As a result, competition for resources, indirect damage through pollution, climate change and spread of invasive species are likely to intensify throughout the 21st Century placing greater pressure on animal populations [1,2]. Currently, there are 10,739 (14.43 %) vertebrate species being classified as threatened (Critically Endangered, Endangered, or Vulnerable) according to the International Union for Conservation of Nature (IUCN) [4], with many

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subspecies and populations additionally at risk of regional extinction. Loss of biodiversity can result in ecosystem collapse, with negative consequences for local human communities and the ecosystem services on which we rely [2]. For these threatened species, small population sizes may result in poor genetic diversity, which reduces a population's ability to adapt to changing environments [5] and further intensifies pressure on species survival. The conservation of wild animal populations is, therefore, a major concern for conservation organisations globally [2].

### 1.1. Conservation of managed wildlife populations

Zoos and aquaria have responded to the current extinction crisis by establishing ex situ populations. Ideally, these populations should remain self-sustaining and genetically stable for potential application in future conservation activities, such as release to the wild [5–7]. As a result, zoos have selected specific, often threatened, species for long-term breeding and management programmes, which aim to maintain population viability and genetic diversity [8]. Examples of these programmes include the Association of Zoos and Aquariums' [9,10] Species Survival Plans (SSPs) and the European Association of Zoos and Aquaria's [11] Ex situ Programmes (EEPs). These programmes aim to maintain 90 % genetic diversity over the next 200 years (sometimes shortened to 100 years) [10]. Genetic diversity is correlated with population size: it is easier to maintain genetic diversity in a larger population, and as such many zoos aim to increase population sizes.

The space available for threatened species in zoos and aquaria is limited, and allocating more space for one species is often at the expense of another [12]. Furthermore, many zoos have increased habitat size to improve animal welfare resulting in fewer habitats and, therefore, a lower carrying capacity [13]. This is evident through zoo records, which show that the average number of animal species per zoo has significantly dropped, with major declines for bird species [14]. Many managed populations are not projected to reach genetic diversity thresholds: Lees and Wilcken [8], for example, demonstrated that only 55 % of their sampled breeding programmes were reaching genetic diversity requirements. Reasons for failure to reach genetic aims include small population sizes, poor reproductive rates, lack of genetic knowledge and challenges with animal transfers [8,13–17]. Over time, some domestic animal breed populations have also become threatened as their population sizes dwindle [16]. As a result, there is a need for conservation of both wildlife and domestic animals from an historic, cultural and rare gene perspective [15,17].

### 1.2. Cryobanking of wildlife species

Cryobanking has emerged as a potential tool to support the conservation of threatened species and aid in *ex situ* breeding and management programmes [18]. Animal samples such as sperm, oocytes, embryos, gonadal tissues and somatic cells can be preserved long-term in liquid nitrogen in facilities dedicated to the storage of living cell samples, and subsequently used for assisted reproductive techniques (ARTs), such as artificial insemination or embryo transfer [19]. The term 'biobank' is frequently used in this respect, though it is important to note that biobanking facilities often refer to the storage of non-living tissues, such as hair or blood [18–20]. For this study, the term 'cryobank' is used to denote living cell banks. Combined with ARTs, cryocbankinghas great potential in halting loss of genetic diversity, with the potential to reintegrate lost genetic diversity into small, isolated populations.

Cryopreserved samples are already well integrated into the management of some domestic animals, with a wide range of assisted reproductive techniques available [16]. While cryopreservation is less integrated into the management of wildlife species currently, the amount of research into implementation is increasing [16]. Mammals, primarily charismatic species, have often been the focus of this research; however, ARTs for specific wild bird, fish, and amphibian species have been developed using techniques that can vary significantly from mammal-based methods [6].

Artificial insemination is one of the most commonly applied ARTs, in which frozen sperm are defrosted for use in reproduction [20]. Regular use of cryopreserved sperm in population management has the potential to aid managers of both wildlife and domestic animals in reaching genetic goals, whilst also reducing costs incurred from animal husbandry [6]. An example is the koala (*Phascolarctos cinereus*) where simulations indicate that to achieve the heterozygosity target of 90 % over 100 years, the projected population of 223 individuals could be reduced to 17 individuals if backcrossing with biobanked sperm is applied [7]. Oocytes and embryos also are cryopreserved and have similar conservation potential, though these samples are less commonly utilised compared to sperm [21]. Additionally, gonadal tissues, such as testicular and ovarian tissue, can also be cultivated and preserved: these cells may in future allow renewable development of sperm and oocytes [18].

Somatic tissue/cell cryopreservation has grown steadily and become a focus of many of the newer wildlife cryobanks. Technologies such as somatic cell nuclear transfer (SCNT) have shown some potential for the preservation of valuable individuals and genetics. With SCNT, the nuclear DNA, but not mitochondrial DNA, of individuals lost to a population could be revived using cryopreserved somatic cells [18]. Recent progress in SCNT has occurred in the black-footed ferret (*Mustela nigripes)*, where a 35-year-old cryopreserved somatic cell sample was used to produce a living clone [22]. In this case, the method used was interspecies SCNT (iSCNT), requiring donor oocytes from a domestic ferret (*Mustela putorius)*. Somatic cell technologies have grown to include stem cell induction, and more recently, in vitro gametogenesis. The conversion of somatic cells to iPSCs, which can be differentiated into a range of cell types, including those that are challenging to obtain such as oocytes, is an emerging and promising area [25]. Some of the potential applications of somatic cells and iPSCs are yet to be realised. For example, in 2023 functional oocytes were developed using somatic cell samples from the tail of a male mouse (*Mus musculus)* [26]. It appears, therefore, that samples collected today may be used for techniques that are yet to be developed [27]. While technically challenging, somatic cell technologies offer possibilities for species where population sizes are especially small [23,24], as is true for many endangered species.

### 1.3. Priorities in cryobanking

The benefits of cryopreservation are manifold: these techniques may aid in assessing genetic diversity, maintaining threatened population heterozygosity, introducing new founder genetics from cryopreserved tissues, and reducing costs associated with housing large animal populations [6,28]. However, obtaining and freezing samples can be technically challenging: for example, collection of samples in the wild may be logistically demanding (not least the issue of storing samples collected in the field), and as a result, sampling is often practised at zoos and aquaria which are well placed to provide a readily accessible source of living samples from a vast array of species. Further challenges are associated with prioritising specific sample types and species, as samples are often collected opportunistically when an animal dies or undergoes veterinary procedures. Additionally, the lack of a centralised database to maintain records of samples being stored globally remains a pervasive issue, which should be addressed to avoid duplicate sampling and ensure effective resource usage. The IUCN, who have recently established the Animal Biobanking for Conservation Specialist Group, are aiming to address the challenges of collecting, sharing and banking these samples [29].

One of the greatest challenges faced by conservationists is identifying species that should be cryopreserved. There are millions of extant wildlife species, the majority of which have not been studied in terms of reproductive biology [3,8]. Currently, there is no consensus on criteria for prioritising between-species (e.g., selection between different animal

species, genera, family) or within-species (selection of individuals or subpopulations within a species) cryopreservation measures. While between-species priorities based on threat status (e.g., IUCN) are widely available, they may not incorporate ecosystem health needs (e.g., keystone species) or evolutionary uniqueness (e.g., Evolutionarily Distinct and Globally Endangered [30]). Within a species, it is important to identify whether sample selection is based on mean kinship, genetic diversity or rare genotypes, as this may affect the individual animals selected for sampling. Similarly, there is a need for information on required sample numbers and cell types for long-term population sustainability. One additional challenge is that the reproductive biology of only 0.25 % of approximately 40,000 vertebrate species is well understood [31]: this lack of reproductive knowledge can hamper cryopreservation strategies. Without a shared biobanking strategy, priorities are likely to be reflective of individual organisational needs, thus resulting in an un-unified approach. It is important that current priorities are therefore identified. The purpose of this systematic review is to identify the sampling priorities of wildlife and domestic animal cryopreservation facilities globally through a review of published cryopreservation literature, while also identifying the cell types and numbers of samples that are recommended both between and within species.

## 2. Methods

The systematic review was run in accordance with the updated PRISMA review process [32] and was pre-registered using the Open Science Framework [33]. The following Boolean string was used to identify relevant papers:

(cryo* OR frozen OR bank* OR genom*) AND (priori* OR strateg* OR sample-selection OR advance* OR programme* OR collection) AND (conservation OR biodiversity) AND (animal OR wild* OR zoo OR aqua*) NOT plant.

Three search engines: "Scopus", "Google Scholar" and "Web of Science" were reviewed for relevant publications in cryopreservation. The search dates were set from 1st January 1970 to 1st June 2023 to provide a sample that adequately reflects current practice and priorities in cryopreservation. Searches were conducted on 23rd September 2023. Duplicates were removed from the resulting database before review at title-abstract level. Papers that recorded the cryopreservation priority or strategies used to select samples for cryopreservation were included at full-text search. Studies that related to non-animal samples (e.g., plant, fungal or microbial samples) were excluded. Both domestic (which incorporates livestock, pets and laboratory animals) and wildlife animal species were included, though the two sectors are treated separately for analysis, as wildlife and domestic animal studies are likely to have different priorities and focuses. We did not differentiate between wildlife species housed in zoos versus those collected in the wild.

For each publication identified, the following additional information was collected: journal, year of publication, authors and affiliation, DOI, organisation, organisation type/field (wildlife, domestic, or mixed) species (if multiple species, the highest taxonomic level(s) was recorded, e.g., Carnivora), the sample type (e.g., sperm, oocyte, embryo, ovarian or testicular tissue, somatic cell, induced pluripotent stem cell), number of samples, and priorities for sample selection (Table 1). It should be noted that while embryos and induced pluripotent stem cells are not collected samples (as they are produced in vitro), these were included in the review as they demonstrate how existing samples are being used to produce new tissue types. If papers did not contain all of the variables above, they were still incorporated into the dataset. Cryopreservation priorities were recorded descriptively, these descriptions were later categorised into themes (see Table 1). These studies were separated into between-species (e.g., selection between different animal species, genera, family) or within-species (selection of individuals or subpopulations within a species) studies. The priorities were identified as specifically as possible: for example, if a study identified mean kinship as a priority, it was labelled as such, rather than the more generic 'genetic diversity'.

**Table 1**
Cryopreservation priorities between and within species.

| Category | Explanation | Within species or between species? |
|---|---|---|
| Threat status | Preservation of species based on threat status(e.g., prioritising IUCN-threatened species over non-threatened species). | Between |
| Evolutionary distinct | Selection of species based on their lack of extant evolutionarily close relatives. | |
| Species diversity | Selection of species to increase taxonomic diversity in cryobanks. | |
| Studbook | Preferential selection of species that feature in existing *ex situ* breeding programmes. | |
| Convenience | Selection of local or easily obtainable animals. | Both |
| Genetic diversity | Selection based on maintaining genetic diversity. | |
| Knowledge acquisition | Selection in order to fill gaps in knowledge of reproductive technology and cryopreservation. | |
| Not stated | No explanation of the selection method. | |
| Revival of Species | Revival of lost genetic diversity in threatened species, or revival of extinct species. | |
| Breed preservation | Selection of specific breeds of domestic animals that are of cultural, genetic or economic interest. | Within |
| Economy | Methods to maximise profitability, including use of cryopreservation to reduce costs. | |
| Mean kinship | Selection to reduce mean kinship. | |
| Pedigree and Founders | Selection based on founders of populations or to maintain a pedigree. | |
| Trait preservation | Conservation of specific animal traits such as milk yield or leg length, rather than an entire breed. | |

### 2.1. Data analysis

Data were collated and figures were prepared using Microsoft Excel™ 2016 and analysis was run using IBM SPSS. Descriptive statistics were used to identify the most common priorities in cryopreservation. A Spearman's correlation was used to identify the relationship between the year of publication and the number of published cryopreservation studies.

## 3. Results

### 3.1. General findings

Initial Boolean string searches revealed 13,287 papers (Fig. 1), of which 3106 duplicates were removed before screening. Following screening, a total of 794 papers were selected for inclusion in the systematic review, with 820 studies identified (26 papers incorporated multiple studies). The majority of studies examined cryopreservation priorities at a within-species level (604 studies, 73.68 %); the remainder investigated priorities between species (216 studies, 26.32 %).

The number of cryopreservation studies published per year was identified (Fig. 2). There was a significant, strong positive correlation between the year of publication and the number of studies published per year ($r_{(s)}= 0.850$, $P < 0.001$), which indicates that significantly more publications of cryopreservation are being published each year.

A total of 264 individual journal and book publishers were identified by the systematic review. The journals *Theriogenology Wild* and *Cryobiology* were identified as publishing the greatest number of studies. A total of 235 organisations were identified as the lead affiliation in cryopreservation papers: the locations of the top ten publishing

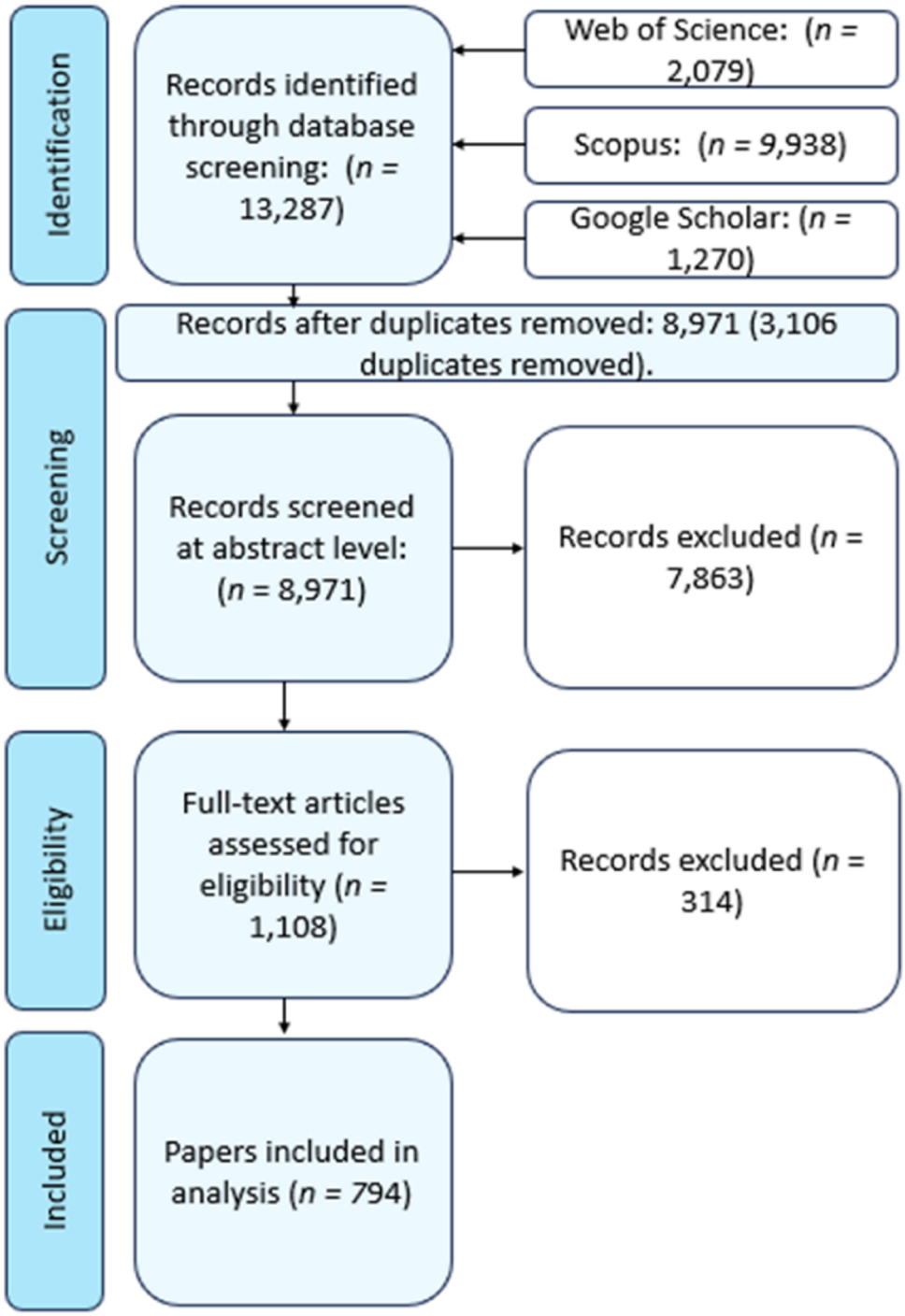


**Fig. 1.** Flow chart depicting the PRISMA process used for this study.

organisations are shown pictorially (Fig. 3).

### 3.2. Wildlife studies

#### 3.2.1. Priorities

A total of 525 wildlife and mixed studies were identified. The priorities stated in published studies are summarised in Fig. 4. Overall, threat status (135 studies, 25.71 %), convenience sampling (121 studies, 23.05 %), and genetic diversity (92 studies, 22.10 %) featured as the three most common priority methods stated.

Irrespective of taxa, only 50 papers reported a recommended number of individuals to be cryopreserved: the average recommendation was for 81 (+/- 76.98 sd) individuals. The recommended number of samples (in which one individual could be banked multiple times) per species was rarely reported in published studies: only 32 studies reported the number of samples to be cryopreserved. The recommended number of samples varied between 6 and 2500, with an average of 277 (+/-854.54 sd) banked samples.

The number of times each cell type featured in cryopreservation studies is summarised in Fig. 5. The most frequently featured cell type was sperm, which appeared almost three times more often than the next most common cell type. Oocytes, embryos and somatic cells featured regularly in studies. The categories were not mutually exclusive; in that a study could encompass several cell types simultaneously.

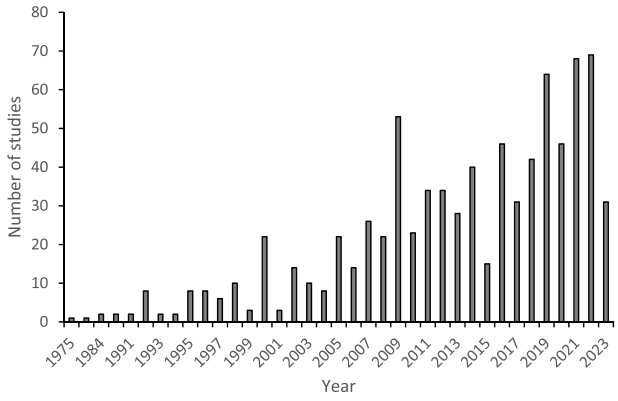


**Fig. 2.** Number of cryopreservation studies published per year (n = 794).

### 3.2.2. Taxonomy

The number of studies identified per taxonomic Class (which appeared in more than five studies) is shown in Fig. 6. Mammals were the focus of the greatest number of studies (213 studies, 40.57 %). A total of 157 genera were identified as being the subject of studies. The most commonly appearing wildlife genera were *Panthera* (12 studies), *Mustela* (8 studies), *Rana* (7 studies) and *Ceratotherium* (6 studies).

### 3.3. Comparison with domestic animals

A total of 295 (35.97 %) domestic animal studies were identified in the systematic review. The most commonly mentioned domestic animal genera were *Bos* (31 studies), *Gallus* (26 studies), *Capra* (21 studies), *Canis* and *Ovis* (17 studies each). In domestic animal studies, the most common priority was to select based on convenience (88 studies, 29.83 %) (Fig. 7). Genetic diversity (70 studies, 23.73 %) and breed preservation (66 studies, 22.38 %) featured as the second and third most common priorities.

## 4. Discussion

This systematic review identified that the most cited wildlife cryopreservation priority was to select species based on threat status. Convenience sampling and genetic diversity also featured commonly as priorities. A range of cell types are featured in the literature, though sperm continues to be the most commonly preserved sample type for captive animals. Surprisingly, there was no unanimous direction for the number of samples to collect per species. Overall, the number of studies published per year on wildlife cryopreservation priorities is increasing

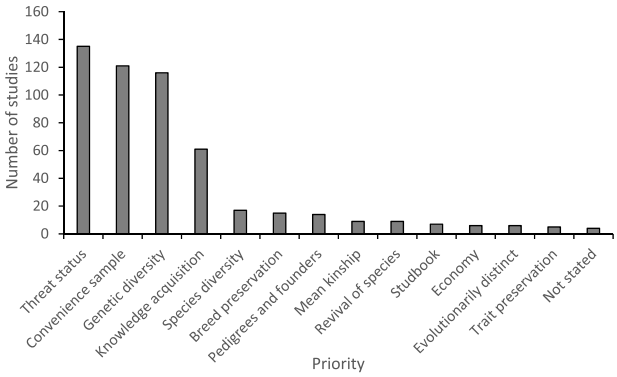


**Fig. 4.** Stated priorities for cryopreservation sampling in wildlife studies (n = 525).

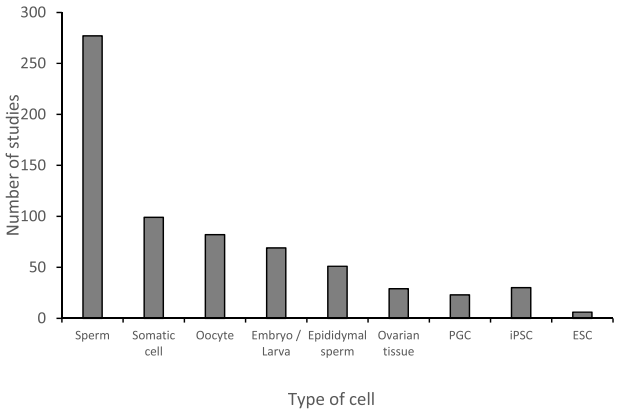


**Fig. 5.** Cell types featuring in wildlife cryopreservation studies (n = 525). ESC, iPSC and PGC refer to embryonic stem cell, induced pluripotent stem cell and primordial germ cell, respectively. Please note that iPSCs are not collected, but rather are converted from other cell types, such as somatic cells.

**Fig. 3.** Top ten locations identified in terms of number of studies. The size of the blue dot indicates the number of studies per location. Location of the blue dot indicates the affiliation of the first author of the study.

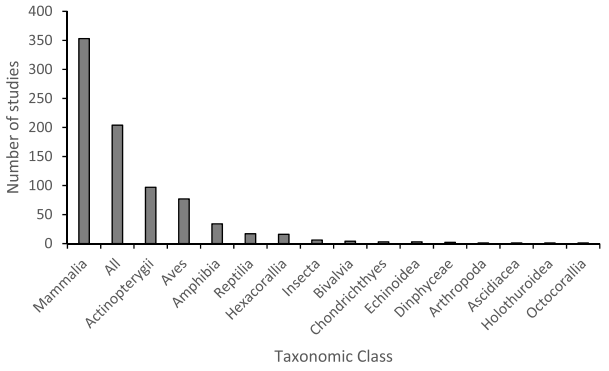


**Fig. 6.** Number of studies per taxonomic group in wildlife cryopreservation studies (n = 525).

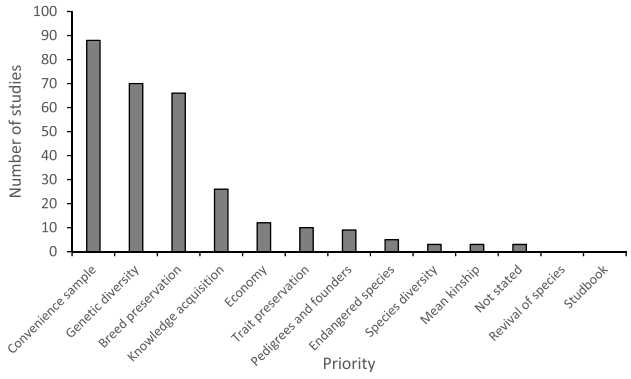


**Fig. 7.** Stated cryopreservation priorities for domestic animals, based on study outputs (n = 295).

over time, suggesting that this topic is receiving greater scientific interest, reflecting growing concerns over the need to conserve genetic diversity for future wildlife conservation efforts.

### 4.1. Wildlife cryobanking priorities

The most common priority between species in the literature was the threat level of the species. While this is an important selection criterion for cryobanks to focus on in light of the rapid loss of biodiversity globally, it may result in species being overlooked while their genetic diversity is high. By the time an animal is endangered, the potential for reduced genetic diversity and limited genetic value of the sample increases [35–39]. From an endangered species standpoint, samples collected now are likely to represent much greater genetic diversity than those collected in 50 years' time [6]. As such, it is important to collect samples from non-priority species classified as Near Threatened or Vulnerable, while genetic diversity is still high. Placing emphasis on animals with a decreasing population trend may have merit in saving genetic diversity for future application. Further, the focus on threat status as the main priority may also overcast other criteria such as evolutionary distinctness, taxonomic diversity or ecosystem value as a keystone species [40].

When selecting within a species, the most frequently cited priority was genetic diversity, a necessary factor for long-term species sustainability. It is another critical priority in cryobanking due to the value of reintroducing alleles back into a population that may become lost over time [7]. However, the guidance for genetic diversity sampling is not always explicitly clear. For example, assessing genetic diversity based on nuclear or mitochondrial DNA is likely to result in differing outcomes [41]. Genetic diversity data, even in *ex situ* breeding and management programmes, are not always available, thus despite the possible improvements to management practices when selecting based on genetic diversity, delays resulting from genetic analysis timelines can be detrimental. Some authors have elaborated by providing clearly quantifiable alternative priorities such as founder representation or mean kinship [34]. Since these metrics do not require genetic testing, they can allow researchers to identify key individuals for sampling with little analytical input other than access to a cooperatively managed breeding programme [166]. Simulations that identify the impact of different sampling approaches would help in providing clearer guidance on the most efficient sampling strategies [7,34].

Knowledge acquisition was cited as a key reason for undertaking cryobanking efforts [42]. This prioritisation technique may focus on taxa that are less represented in the wider literature base. While some agricultural and laboratory species are now reasonably well studied, there remain major logistical challenges for some taxa, such as birds and reptiles, that have limited the wider use of cryobanking [45–48]. Additionally, reproductive physiology and chill sensitivity differ considerably even between closely related species and individuals, highlighting the importance of continued expansion of our knowledge base for future cryobanking [49]. Therefore, studies on non-endangered species are critical so that cryopreservation techniques and associated ARTs are developed and ready to be extrapolated to other species.

### 4.2. Taxonomic focus in cryobanking

Overall, mammals were the most featured taxonomic class in cryobanking priority papers, a focus that has been identified in other scientific fields, such as in conservation translocations [42], zoo research [43] and in the public's preferences for species [44]. From a functional standpoint, aside from humans, cryobanking and ARTs have been most extensively developed for farmed and laboratory mammal species, therefore, it is unsurprising that mammals featured as the most common taxonomic group. Researchers face fewer biological and technical hurdles when studying mammalian reproductive physiology, as methods can be adapted more easily from bovine and mouse reproduction studies [45].

Several vertebrate classes featured rarely in cryobanking, including Reptilia and Chondrichthyes (sharks and rays). These taxa are not traditionally farmed and may pose technical challenges for undertaking research on reproductive physiology and development of cryopreservation protocols due to a lack of access to study animals or related managed species as models, thereby impacting the establishment of assisted reproductive techniques [46,47].

Invertebrates also featured rarely in the cryopreservation priority literature, though promising initial research is available, especially for corals and some insects [46]. These studies may have great potential in providing an evidence base for more widespread banking of these taxa. Special focus, for example, has been applied to coral gamete cryopreservation given the imminent threats to these animals associated with coral bleaching [46]. Given that at least 95.5 % of all animal species are invertebrates [4], there remain many taxa for which cryopreservation protocols are not yet developed.

### 4.3. Cell types in the cryobank

Overall, sperm was the most frequently discussed cell type identified in cryopreservation literature. From an historical perspective, a focus on sperm preservation is intuitive, as sperm typically have better cryopreservation success rates and are used for comparatively less complex procedures (e.g., artificial insemination, [48]). More complex techniques, such as SCNT, were not available at the time that early cryopreservation studies were published [49]. While new techniques are available, it appears that sperm remain the main currency in cryopreservation due to their ease of collection for captive animals, and

application in reproductive science [7].

While valuable, banked sperm are a finite cell source. In contrast, other cell types, including stem cells and testicular tissue, can be propagated under laboratory conditions [47]. There is also comparatively less focus on preserving female genetic material, such as ovarian tissue or oocytes. A future direction for the cryopreservation community is to capture female genetics along with male genetics. Oocytes are known to be challenging to cryopreserve due to their large size, and in many species, large lipid reserves, in comparison to sperm, which complicate freezing processes [18]. The potential value of these tissues is significant: when combined with sperm in the laboratory, they can be used to generate embryos that have novel genetic combinations.

Somatic cells feature in the literature as the second most common cell type, with stem cells (including induced pluripotent stem cells) featuring less frequently. It should be noted that somatic cell samples may contain naïve stem cells for harvesting or could be converted to stem cells in the laboratory. Stem cells have great potential in future cryopreservation ventures, as they can be differentiated into other cell types, such as oocytes [26]. While this technology is in its infancy and proof of concept has been shown only for select species, there may be application of this technique in endangered species, for whom oocytes are challenging to obtain. Somatic and stem cells can be collected even from infertile and recently deceased individuals, and some cell types, such as fibroblasts, are reasonably tolerant of freezing [25]. Further investment into stem cell science is required to bridge the knowledge gap between taxa [48,49].

### 4.4. Domestic animals

Overall, wildlife and mixed studies featured in the cryopreservation literature more frequently than domestic animal studies based on the selection criteria used. This discrepancy may stem from differences between organisations: domestic cryopreservation studies are often run in association with commercial farms [17], that may be interested in gaining results for internal application, rather than sharing externally. Zoos and aquaria, by contrast, are required to collaborate in order to maintain species populations [7], and there may be more incentive to share success through publications. Given this difference in objectives, direct comparison of the priorities for domestic animal research (which often focus on breed and trait preservation) and wildlife studies, which tend to focus on endangered species and genetic diversity preservation, is not recommended.

Generally, domestic animal studies tended to focus on only a handful of genera, such as *Gallus* and *Bos*. This focus on specific genera may have considerable value when applied to wildlife species. Fundamental studies on techniques are often more feasible for domestic animals, on account of larger population sizes and animal accessibility. The research outcomes can then be adapted to closely related species [50]. For example, research in the domestic horse (*Equus caballus*) and donkey *(Equus asinus*) have been used to better understand reproductive physiology and cryobiology not only in these domestic species, but also in the Critically Endangered Przewalski's wild horse (*E. przewalskii)* [51] and Persian onager [52], for which there has been recent ART success.

### 4.5. Considerations and future directions

As with any systematic review, there are several limitations which must be considered. First, the review was undertaken on UK servers, with the searches being undertaken in English. This may result in a bias toward cryopreservation studies undertaken in the UK and other English-speaking studies. Future studies could address this by conducting searches using multiple languages. It should also be noted that the cryopreservation priorities were identified using published literature. The results identify species featured only in published cryopreservation literature, rather than a true reflection of the species that are being banked. Cryobanks such as San Diego Zoo's Frozen Zoo [42] are sampling a much wider variety of species than were identified by this systematic review; many of their samples are collected as a matter of course and, as such, are not recorded in publications. Furthermore, many cryobanks are relatively new and have not yet published protocols or findings. To capture the full extent of cryobanking outside the published literature, surveying these facilities is necessary (e.g., see Mooney et al., [42]). It would also be beneficial for cryobanks to adopt a standardised data sharing protocol, to enable greater discoverability of samples between individual cryobanks.

We also note that we did not differentiate between samples taken from wildlife housed in a captive setting versus those from the wild. It is likely that there is a difference between the types of samples collected from wild and captive animals, with reproductive tissues such as sperm and oocytes being more challenging to collect in the wild, except from cadavers [25]. Thus, differences in sampling priorities between wild and captive animals are expected in terms of tissue types and individuals. Numerous additional challenges are faced when obtaining samples from animals in the wild, including safe methods for obtaining samples, individual animal identification, and freezing and storage methods under field conditions. Care should also be given to access and benefit sharing when sampling local wildlife species, for example through the Nagoya protocol [53]. Future studies should aim to differentiate between wild and captive animal sampling in order to identify whether their priorities diverge.

Consideration must also be given to the ethics associated with cryopreservation and assisted reproductive techniques [50]. For example, the success rates associated with SCNT are typically low, and interspecies SCNT especially so. The real-world implication of poor viability is that numerous trials will be needed to produce only a few healthy births, with the remaining pregnancies ending in pre- and post-natal loss. Additionally, interspecies SCNT typically has higher success rates between more closely related donor-recipient species with the same genus generally having the best results [51]. This means that closely related, potentially also threatened species, may be required as surrogates. Ethics must be considered carefully before engaging in any studies of this kind.

One other reproductive technique in need of ethical consideration is the use of xenografting for in vivo gamete production. In reproductive xenografting, gonadal tissues from one species are transferred to another species [50]. While primarily trialled in laboratory species and poultry, this technique could be used to generate gametes from endangered species. For example, xenografting of both deer and bison testicular tissue into recipient mice has already resulted in successful spermatogenesis [50]. While a promising tool to generate cells for threatened species, consideration should be given to the ethical consequences for recipient animals, such as the impact of immunosuppressants or gene editing on health.

At the inception of cell cryopreservation, the potential applications of frozen samples were not fully realised, and it is likely that some of the future uses of cryopreserved cells are still not known [18]. With the continued advancements in media and equipment development and cryopreservation techniques, post-thaw viability and ART success rates will improve, technical costs will decrease, and new applications for cryopreservation will become available. As species become increasingly endangered or extinct as the technology and practices mature, it is essential that cryopreservation priorities are identified, mutually agreed upon, and implemented now, so that the relevant samples can be collected.

The results of this study demonstrate that there is a need for greater collaboration between global cryobanks. Overall, many cryobanks may be focusing on institution-specific priority projects and species, which may have resulted in limited collaboration between organisations. This results in limited knowledge-sharing between facilities, and the contents of each cryobank may not be known to other facilities [42]. Communication and collaboration between cryobanks have several major benefits, including duplication of important samples across cryobanks, so

that if an accident occurs at one facility there remains a viable backup elsewhere [54]. Communication could also aid cryobanks in obtaining important samples, particularly when cryobanks are located in different countries and legislation reduces movement of samples across trade boundaries (e.g., Convention on the International Trade of Endangered Species of fauna and flora, CITES). A collaborative approach could reduce duplication of effort and maximise the potential for collection of important samples, particularly those that could be applied in conservation breeding scenarios. Collaboration could be facilitated through partnerships between individual cryobanks, or more widely through umbrella organisations such as the IUCN SSC Animal Biobanking for Conservation Specialist Group [29]. Part of this collaboration could include sharing of knowledge of the samples stored at each cryobank, similar to the Zoological Information Management System (ZIMS) programme used by zoos globally [55]. This could also involve the identification of priority sample types and species for all subscribing cryobanks. These approaches could help reduce missed opportunities for sampling animals that had been accessible to researchers, but are now entirely lost, such as the now-extinct Yangtze river dolphin (*Lipotes vexillifer*) [56].

## 5. Conclusion

Threat status has emerged as the strongest priority in wildlife cryopreservation, though genetic diversity, convenience sampling and knowledge acquisition also appear as key themes. There is no clear consensus on the numbers of samples that need to be banked per species, nor the number of individuals that should be represented. These discrepancies are likely a result of the different missions of cryopreservation organisations globally, which may be focusing on anything from wildlife cryopreservation to livestock profitability. From a wildlife standpoint, a focus on saving a wider diversity of tissue types, especially female reproductive tissues, somatic cells, and species, may be useful to ensure that cryobanks are in a good position to support future species survival. While the focus on threatened species is valuable, greater emphasis on including Vulnerable or Near Threatened populations amongst the banked samples would ensure maintenance of genetic diversity before it is lost. There is a need for greater collaboration between cryobanks so that priorities and resources are shared, the acquisition of important samples is streamlined, and important samples can be stored at multiple facilities as a safety precaution. This collaboration could be facilitated through the role of developing groups, such as the IUCN SSC's Animal Biobanking for Conservation Specialist Group. Cryobanks have the potential to accelerate conservation impact, as banked samples are likely to accrue interest in terms of their value as genetic diversity of species erodes. As cryobanks represent a last safeguard against loss of animal populations, the consequences of not banking key species could well be extinction.

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## CRediT authorship contribution statement

**Mastromonaco Gabriela F:** Writing – review & editing, Validation, Supervision. **Wilson Philippe:** Writing – review & editing, Supervision. **Mooney Andrew:** Writing – review & editing, Writing – original draft, Supervision, Methodology. **Walker Susan L:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Spooner Sarah Louise:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology. **Brereton James Edward:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **White Samuel:** Writing – review & editing, Writing – original draft, Supervision, Methodology. **Hunter Elena:** Writing – review & editing, Supervision.

## Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no part of this manuscript was developed using generative AI

## Declaration of Competing Interest

The authors declare no competing interests.

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## References

[1] United Nations. World population prospects - population division - United Nations. ⟨https://population.un.org/wpp/⟩ (accessed March 21, 2023).
[2] A. Treves, R.B. Wallace, L. Naughton-Treves, A. Morales, Co-managing human–wildlife conflicts: a review, Human. Dimens. Wildl. 11 (2006) 383–396, https://doi.org/10.1080/10871200600984265.
[3] A.D. Barnosky, N. Matzke, S. Tomiya, G.O.U. Wogan, B. Swartz, T.B. Quental, et al., Has the earth's sixth mass extinction already arrived? Nature 471 (2012) 51–57, https://doi.org/10.1038/nature09678.
[4] IUCN. The IUCN red list of threatened species. IUCN red list of threatened species 2023. ⟨https://www.iucnredlist.org/en⟩ (accessed March 28, 2023).
[5] K. Ralls, P. Sunnucks, R.C. Lacy, R. Frankham, Genetic rescue: a critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation, Biol. Cons. 251 (2020) 108784, https://doi.org/10.1016/j.biocon.2020.108784.
[6] J.D. Ballou, R.C. Lacy, K. Traylor-Holzer, J. Bauman, J.A. Ivy, C. Asa, Strategies for establishing and using genome resource banks to protect genetic diversity in conservation breeding programs, Zoo. Biol. 2022 (2022) zoo.21741, https://doi.org/10.1002/zoo.21741.
[7] L.G. Howell, S.D. Johnston, J.K. O'Brien, R. Frankham, J.C. Rodger, S.A. Ryan, et al., Modelling genetic benefits and financial costs of integrating biobanking into the captive management of Koalas, Animals 12 (2022) 990, https://doi.org/10.3390/ani12080990.
[8] C.M. Lees, J. Wilcken, Sustaining the Ark: the challenges faced by zoos in maintaining viable populations, Int. Zoo. Yearb. 43 (2009) 6–18, https://doi.org/10.1111/j.1748-1090.2008.00066.x.
[9] AZA. Species Survival Programmes 2022. ⟨https://www.aza.org/species-survival-plan-programs?locale=en⟩ (accessed on March 31, 2023).
[10] L. Wilson, C. Dorsey, D. Moore, Challenges and solutions: An analysis of community-reported needs of AZA collaboratively managed animal populations, Zoo. Biol. 38 (2019) 45–54, https://doi.org/10.1002/zoo.21467.
[11] EAZA. EAZA Ex situ Programme pages. 2022. ⟨https://www.eaza.net/conservation/programmes/eep-pages/⟩ (accessed on March 31, 2023).
[12] C. McCann, D.M. Powell, Is there any more room on the Ark? An analysis of space allocation in four mammalian taxa, Zoo. Biol. 38 (2019) 36–44, https://doi.org/10.1002/zoo.21469.
[13] D.M. Powell, C.L. Dorsey, L.J. Faust, Advancing the science behind animal program sustainability: an overview of the special issue, Zoo. Biol. 38 (2019) 5–11, https://doi.org/10.1002/zoo.21474.
[14] S.R. Brereton, J.E. Brereton, Sixty years of collection planning: what species do zoos and aquariums keep? Int. Zoo. Yearb. 54 (2020) 131–145, https://doi.org/10.1111/izy.12264.
[15] D.P. Sponenberg, Conserving the genetic diversity of domesticated livestock, Diversity 12 (2020) 282, https://doi.org/10.3390/d12070282.
[16] H.E. Jones, P.B. Wilson, Progress and opportunities through use of genomics in animal production, Trends Genet. 38 (2022) 1228–1252, https://doi.org/10.1016/j.tig.2022.06.014.
[17] W. Wainwright, A.B. Vosough, A. Mcvittie, G. Simm, D. Moran, Prioritising support for cost effective rare breed conservation using multi-criteria decision analysis, Front. Ecol. Evol. 7 (2019). ⟨https://www.frontiersin.org/articles/10.3389/fevo.2019.00110⟩.
[18] R.J. Hobbs, J.K. O'Brien, R.E. Spindler, Strategic gene banking for conservation: the ins and outs of a living bank, in: A.B. Kaufman, M.J. Bashaw, T.L. Maple (Eds.), Scientific Foundations of Zoos and Aquariums, 1st ed., Cambridge University Press, 2019, pp. 112–146, https://doi.org/10.1017/9781108183147.005.
[19] G. Leroy, P. Boettcher, B. Besbes, C. Danchin-Burge, R. Baumung, S.J. Hiemstra, Cryoconservation of animal genetic resources in Europe and Two African countries: a gap analysis, Diversity 11 (2019) 240, https://doi.org/10.3390/d11120240.
[20] R.L. Bolton, A. Mooney, M.T. Pettit, A.E. Bolton, L. Morgan, G.J. Drake, R. Appeltant, S.L. Walker, J.D. Gills, C. Hvilsom, Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking, Reprod. Fertil. 3

(2022) 121–146. ⟨https://raf.bioscientifica.com/view/journals/raf/3/3/RAF-22-0005.xml⟩.
[21] B.S. Shapiro, S.T. Daneshmand, F.C. Garner, M. Aguirre, C. Hudson, Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer, Fertil. Steril. 102 (2014) 3–9, https://doi.org/10.1016/j.fertnstert.2014.04.018.
[22] R. Fritts, Cloning goes wild, Science 375 (2022) 134–137, https://doi.org/10.1126/science.acz9982.
[23] I. Lagutina, H. Fulka, G. Lazzari, C. Galli, Interspecies somatic cell nuclear transfer: advancements and problems, Cell. Reprogram. 15 (2013) 374–384, https://doi.org/10.1089/cell.2013.0036.
[24] C.E. Pope, M.C. Gómez, B.L. Dresser, In vitro production and transfer of cat embryos in the 21st century, Theriogenology 66 (2006) 59–71, https://doi.org/10.1016/j.theriogenology.2006.03.014.
[25] E.A. Praxedes, N.L.B. De Queiroz, A.A. Borges, M.B. Silva, M.V.O. Santos, L. R. Ribeiro, et al., Quantitative and descriptive histological aspects of jaguar (Panthera onca Linnaeus, 1758) ear skin as a step towards formation of biobanks, Anat. Histol. Embryol. 49 (2020) 121–129, https://doi.org/10.1111/ahe.12500.
[26] K. Murakami, N. Hamazaki, N. Hamada, G. Nagamatsu, I. Okamoto, H. Ohta, et al., Generation of functional oocytes from male mice in vitro, Nature 615 (2023) 900–906, https://doi.org/10.1038/s41586-023-05834-x.
[27] K. Benirschke, The frozen zoo concept, Zoo. Biol. 3 (1984) 325–328, https://doi.org/10.1002/zoo.1430030405.
[28] O.A. Ryder, A. McLaren, S. Brenner, Y.P. Zhang, K. Benirschke, DNA banks for endangered animal species, Science 288 (2000) 275–277, https://doi.org/10.1126/science.288.5464.275.
[29] IUCN. New specialist group aims to establish a global network of biobanking for conservation. 2023. ⟨https://www.iucn.org/story/202304/new-specialist-group-aims-establish-global-network-biobanking-conservation⟩.
[30] EDGE. What is EDGE? EDGE of Existence 2022. ⟨https://www.edgeofexistence.org/what-is-edge/⟩ (accessed March 28, 2023).
[31] P. Comizzoli, Biobanking efforts and new advances in male fertility preservation for rare and endangered species, Asian J. Androl. 17 (2015) 640–645, https://doi.org/10.4103/1008-682X.153849.
[32] A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gotzsche, J.P.A. Ioannidis, et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration, BMJ 339 (2009) 2700–2710, https://doi.org/10.1136/bmj.b2700.
[33] J.E. Brereton, S.L. Walker, A. Mooney, P.B. Wilson, S. White, Systematic review of cryoconservation priorities, Open Sci. Framew. (2023). ⟨https://osf.io/5qetf/resources⟩.
[34] L.A. Johnston, R.C. Lacy, Genome resource banking for species conservation: selection of sperm donors, Cryobiology 32 (1995) 68–77, https://doi.org/10.1006/cryo.1995.1006.
[35] I.W. Rowlands, Artificial insemination of mammals in captivity, Int. Zoo. Yearb. 14 (1974) 230–233, https://doi.org/10.1111/j.1748-1090.1974.tb00834.x.
[36] M.T. Prieto, M.J. Sanchez-Calabuig, T.B. Hildebrandt, A. Santiago-Moreno, J. Saragusty, Sperm cryopreservation in wild animals, Eur. J. Wildl. Res. 60 (2014) 851–864, https://doi.org/10.1007/s10344-014-0858-4.
[37] J. Clulow, S. Clulow, Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed, Reprod. Fertil. Dev. 28 (2016) 1116, https://doi.org/10.1071/RD15466.
[38] S.M. Hopkins, D.L. Armstrong, S.K.C. Hummel, s. Junior, Successful cryopreservation of Gaur (Bos gaurus) epididymal spermatozoa, J. Zoo. Anim. Med. 19 (1988) 195–201, https://doi.org/10.2307/20094887.
[39] J. Santiago-Moreno, M. Coloma, A. Toledano-Díaz, C. Castaño, A. Gómez-Brunet, A. López-Sebastián, Assisted reproduction in Mediterranean wild ruminants: lessons from the Spanish ibex (Capra pyrenaica), Reprod. Domest. Rumin. 7 (2011) 431–439.
[40] A. Mooney, O.A. Ryder, M.L. Houck, J. Staerk, D.A. Conde, Y.M. Buckley, Maximizing the potential for living cell banks to contribute to global conservation priorities, Zoo. Biol. (2023) zoo.21787, https://doi.org/10.1002/zoo.21787.
[41] V.B. Cowl, P. Comizzoli, R. Appeltant, R.L. Bolton, R. Browne, W.V. Holt, S. L. Walker, S.A. Williams, Cloning for the twenty-first century and its place in endangered species conservation, Annu. Rev. Anim. Biosci. 12 (2023).
[42] B. Bajomi, A.S. Pullin, G.B. Stewart, A. Takács-Sánta, Bias and dispersal in the animal reintroduction literature, Oryx 44 (2010) 358–365, https://doi.org/10.1017/S0030605310000281.
[43] V.A. Melfi, There are big gaps in our knowledge, and thus approach, to zoo animal welfare: a case for evidence-based zoo animal management, Zoo. Biol. 28 (2009), https://doi.org/10.1002/zoo.20288.
[44] C. Albert, G.M. Luque, F. Courchamp, The twenty most charismatic species, PLOS One 13 (2018) 0199149, https://doi.org/10.1371/journal.pone.0199149.
[45] P. Comizzoli, N. Songsasen, D.E. Wildt, Protecting and extending fertility for females of wild and endangered mammals, in: T.K. Woodruff, L. Zoloth, L. Campo-Engelstein, S. Rodriguez (Eds.), Oncofertility: Ethical, Legal, Social, and Medical Perspectives, Springer US, Boston, MA, 2010, pp. 87–100, https://doi.org/10.1007/978-1-4419-6518-9_7.
[46] M. Hagedorn, R. Spindler, The reality, use and potential for cryopreservation of coral reefs, in: W.V. Holt, J.L. Brown, P. Comizzoli (Eds.), Reproductive Sciences in Animal Conservation: Progress and Prospects, Springer, New York, NY, 2014, pp. 317–329, https://doi.org/10.1007/978-1-4939-0820-2_13.
[47] R. Upton, N.E. Calatayud, S. Clulow, D. Brett, A.L. Burton, K. Colyvas, J. Clulow, Refrigerated storage and cryopreservation of hormonally induced sperm in the threatened frog, Litoria aurea, Anim. Reprod. Sci. 262 (2024) 107416.
[48] C. Lambo, R. Grahn, L. Lyons, H. Bateman, J. Newsom, W. Swanson, Comparative fertility of freshly collected vs frozen–thawed semen with laparoscopic oviductal artificial insemination in domestic cats, Reprod. Domest. Anim. 47 (2012) 284–288, https://doi.org/10.1111/rda.12038.
[49] G. Mastromonaco, L.A. González-Grajales, M. Filice, Comizzoli, Somatic cells, stem cells, and induced pluripotent stem cells: how do they now contribute to conservation? Reprod. Sci. Anim. Conserv.: Prog. Prospects (2014) 385–427.
[50] A. Abbasi, Honaramooz, Salvaging genetic material from endangered species: xenografting of testis tissue from immature bison and deer donors into recipient mice results in complete spermatogenesis, Biol. Reprod. 85 (2011), https://doi.org/10.1093/biolreprod/85.s1.174.
[51] B.J. Novak, O.A. Ryder, M.L. Houck, A.S. Putnam, K. Walker, L. Russell, B. Russell, S. Walker, S.S. Arenivas, L. Aston, G. Veneklasen, J.A. Ivy, K.P. Koepfli, A. Rusnak, J. Simek, A. Zhuk, R. Phelan, R. Endangered Przewalski's horse, Equus przewalskii, cloned from historically cryopreserved cells. bioRxiv, 2023. 12. ⟨https://www.biorxiv.org/content/10.1101/2023.12.20.572538v2.abstract⟩.
[52] M.W. Schook, D.E. Wildt, R.B. Weiss, B.A. Wolfe, K.E. Archibald, B.S. Pukazhenthi, Fundamental studies of the reproductive biology of the endangered Persian onager (*Equus hemionus onager*) result in first wild equid offspring from artificial insemination, Biol. Reprod. 89 (2013) 41–51. ⟨https://academic.oup.com/biolreprod/article/89/2/41,%201-13/2514180⟩.
[53] P. Comizzoli, W.V. Holt, Implications of the Nagoya Protocol for genome resource banks composed of biomaterials from rare and endangered species, Reprod. Fertil. Dev. 28 (2016) 1145–1160.
[54] M.G. Larman, S. Hashimoto, Y. Morimoto, D.K. Gardner, Cryopreservation in ART and concerns with contamination during cryobanking, Reprod. Med. Biol. 13 (2014) 107–117. ⟨https://link.springer.com/article/10.1007/s12522-014-0176-2⟩.
[55] S. Poo, S.M. Whitfield, A. Shepack, G.J. Watkins-Colwell, G. Nelson, J. Goodwin, P. Chakrabarty, Bridging the research gap between live collections in zoos and preserved collections in natural history museums, BioScience 72 (2022) 449–460, https://doi.org/10.1093/biosci/biac022.
[56] S.T. Turvey, R.L. Pitman, B.L. Taylor, J. Barlow, T. Akamatsu, L.A. Barrett, D. Wang, First human-caused extinction of a cetacean species? Biol. Lett. 3 (2007) 537–540, https://doi.org/10.1098/rsbl.2007.0292.