REVIEW

Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking

Rhiannon L Bolton[1], **Andrew Mooney**[2], **Matt T Pettit**[1,3], **Anthony E Bolton**[1], **Lucy Morgan**[4], **Gabby J Drake**[5], **Ruth Appeltant**[6], **Susan L Walker**[1,5], **James D Gillis**[7] and **Christina Hvilsom**[8]

[1]Nature's SAFE, Chapel Field Stud, Ash Lane, Whitchurch, Shropshire, UK
[2]Dublin Zoo, Phoenix Park, Dublin 8, Ireland
[3]IMT International Limited, Tattenhall, Chester, UK
[4]Gemini Genetics, Chapel Field Stud, Ash Lane, Whitchurch, UK
[5]Chester Zoo, Upton-by-Chester, UK
[6]Nuffield Department of Women's and Reproductive Health, University of Oxford, Women's Centre, Level 3, John Radcliffe Hospital, Oxford, UK
[7]South-East Zoo Alliance for Reproduction & Conservation, Yulee, Florida, USA
[8]Copenhagen Zoo, Frederiksberg, Denmark

Correspondence should be addressed to R L Bolton: rhiannon@natures-safe.com

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Abstract

Biodiversity is defined as the presence of a variety of living organisms on the Earth that is essential for human survival. However, anthropogenic activities are causing the sixth mass extinction, threatening even our own species. For many animals, dwindling numbers are becoming fragmented populations with low genetic diversity, threatening long-term species viability. With extinction rates 1000–10,000 times greater than natural, *ex situ* and *in situ* conservation programmes need additional support to save species. The indefinite storage of cryopreserved (−196°C) viable cells and tissues (cryobanking), followed by assisted or advanced assisted reproductive technology (ART: utilisation of oocytes and spermatozoa to generate offspring; aART: utilisation of somatic cell genetic material to generate offspring), may be the only hope for species' long-term survival. As such, cryobanking should be considered a necessity for all future conservation strategies. Following cryopreservation, ART/aART can be used to reinstate lost genetics back into a population, resurrecting biodiversity. However, for this to be successful, species-specific protocol optimisation and increased knowledge of basic biology for many taxa are required. Current ART/aART is primarily focused on mammalian taxa; however, this needs to be extended to all, including to some of the most endangered species: amphibians. Gamete, reproductive tissue and somatic cell cryobanking can fill the gap between losing genetic diversity today and future technological developments. This review explores species prioritisation for cryobanking and the successes and challenges of cryopreservation and multiple ARTs/aARTs. We here discuss the value of cryobanking before more species are lost and the potential of advanced reproductive technologies not only to halt but also to reverse biodiversity loss.

Lay summary

The world is undergoing its sixth mass extinction; however, unlike previous events, the latest is caused by human activities and is resulting in the largest loss of biodiversity (all living things on Earth) for 65 million years. With an extinction rate 1000–10,000-fold greater than natural, this catastrophic decline in biodiversity is threatening our own survival. As the number of individuals within a species declines, genetic diversity reduces, threatening their long-term existence. In this review, the authors summarise approaches to indefinitely preserve living cells and tissues at low temperatures (cryobanking) and the technologies required to resurrect biodiversity. In the future when appropriate techniques become available, these living samples can be thawed and used to reinstate genetic diversity and produce live young

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ones of endangered species, enabling their long-term survival. The successes and challenges of genome resource cryopreservation are discussed to enable a move towards a future of stable biodiversity.

Keywords: ▶ biobanking ▶ cryopreservation ▶ biodiversity ▶ assisted reproductive technology ▶ conservation

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Introduction

Humans are causing the sixth mass extinction, the largest predicted loss of biodiversity for 65 million years, with 41% of amphibians, 26% of mammals and 14% of bird species assessed by the International Union for the Conservation of Nature (IUCN) being threatened with extinction (Ceballos *et al.* 2015, Ceballos & Ehrlich 2018, IUCN 2021). The catastrophic decline in biodiversity is a global threat to our own existence, affecting our economies, societal equality and way of life, including the food we eat and our climate (WHO 2015). This current loss of species is estimated to be between 1000- and 10,000-fold higher than the natural extinction rate (Ceballos *et al.* 2015, Turvey & Crees 2019). Human activity is changing the environment too fast for organisms to evolve in response, resulting in extinction (Ceballos & Ehrlich 2018). Restoring habitats alone will not halt the decline in biodiversity as many species are now fragmented, resulting in unviable populations with low genetic diversity (Hoban *et al.* 2020).

Animal conservation aims to maintain populations large enough, and with enough genetic diversity, to be sustainable (Comizzoli *et al.* 2019). *Ex situ* breeding programmes are a vital insurance policy for preserving endangered species and for enabling research, for example, into their behaviour and physiology. In zoos, pedigree-based management typically aims to maintain 90% of genetic diversity over 100 years and minimise mean kinship and inbreeding in threatened populations to retain the evolutionary potential of the species of interest (Ballou *et al.* 2010). Using captive breeding programmes to maintain genetic diversity is not always successful due to lack of reproduction, for example, due to unnatural social structures resulting in reduced breeding behaviour, lack of mate choice or limited number of founders potentially leading to inbreeding (Lees & Wilcken 2009). Furthermore, transporting large animals between different locations for breeding purposes comes with logistical and welfare challenges with the addition of potential disease transmission (Pukazhenthi & Wildt 2004). For some species with unsuccessful breeding programmes (Lees & Wilcken 2009), cryopreserving (freezing cells with cryoprotectants enabling long-term viable cell and tissue storage), followed by cryobanking (indefinite storage of viable cells and tissue in liquid nitrogen at −196°C or ultra-low freezers) and assisted or advanced assisted reproductive technology (ART/aART) can save the genotypes that are being lost today (Mitchell & Williams 2022, for definitions please see Supplementary Table 1, see section on supplementary materials given at the end of this article). ART includes techniques that utilise oocytes and spermatozoa to generate offspring such as artificial insemination (AI), *in vitro* fertilisation (IVF) or intra cytoplasmic sperm injection (ICSI) (Brown *et al.* 2004, Howard *et al.* 2016, Briski & Salamone 2022). These techniques are used for a number of taxa, but species-specific protocols need honing for many endangered species. More recently, the use of aART, technologies that utilise genetic material from somatic cells to generate offspring, has been highlighted as a key technology to resurrect biodiversity such as somatic cell nuclear transfer (SCNT) or induced pluripotent stem cells (iPSC) (Gómez *et al.* 2004, Hikabe *et al.* 2016). Prior to ART or aART, a vital aspect of conservation is storing viable cells and tissues to enable the reintroduction of genes. As a result, genetic diversity can increase within a population, allowing species to recover. This is particularly important for aART, where technology needs further development. Storing viable samples in a biobank not only enables the technology to catch up but also prevents vital genetics from being lost.

A biobank is a repository of biological samples, that is, a searchable, organised collection of biological samples and associated data stored predominantly for research or management, for example, of captive populations (Agca 2012, Hewitt & Watson 2013). Biobanking is not new; conservationists have been collecting samples from wildlife for decades to save genetic diversity (Soulé *et al.* 1985, Montfort 2014). These samples are vital to improve understanding of the fundamental biology of rare and endangered species (Comizzoli & Wildt 2017). Cryopreserving (freezing cells with cryoprotectants enabling long-term viable storage using liquid nitrogen

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at −196°C or ultra-low freezers) then storing cells in a cryobank (biobank of viable, cryopreserved biological samples), to enable the maintenance or regeneration of a species for conservation purposes, is highly specialised in that it requires the application of several complex and novel ARTs working in harmony to be truly effective. Indeed, Comizzoli and Wildt (2017) quote that cryobanking is a 'crucial unfilled gap – offering a backup storage of the extant genomes of living species that are already under threat or are likely to be soon.' As the biodiversity crisis continues, there is an increasing need for global conservation management of endangered species and to interconnect all populations throughout the *ex situ–in situ* continuum to maximise the available genetic diversity. Depending on the species' specific needs, cryobanking could be deemed necessary in the conservation strategy for single or multiple species as part of the One Plan Approach (the One Plan Approach coined by the Conservation Planning Specialist Group of the IUCN; Lees & Wilcken 2011, Byers *et al.* 2013, Traylor-Holzer *et al.* 2018).

Successful maintenance and regeneration of a species are primarily dependent on genetic biodiversity (Choudhary *et al.* 2016). Heterozygosity of a population undoubtedly contributes to stabilisation and robustness of the effective population size (Soulé 1987). Without adequate genetic diversity, any species will inevitably become extinct (Ryder & Onuma 2018). For some species, such as the northern white rhinoceros (*Ceratotherium simum cottoni*), there are already too few individuals remaining to maintain genetic diversity for the long-term sustainability of the population (Korody *et al.* 2021). For these species, aART may be their only hope of long-term survival. For both ART and aART to be successful, there needs to be a knowledge of basic biology, which is lacking for many species (Herrick 2019). Indeed, there is an understanding of the reproductive physiology of only approximately 250 species, with a bias towards mammals and birds, while amphibians are most at risk of extinction (Comizzoli *et al.* 2019, see Case Study: Box 1, Fig. 1). This results in ART and aART developed for specific domestic animals being used as a 'model' for taxonomically similar wild species. One example of ART successfully being applied to selected endangered species is AI. Approaching 100 species of wild mammals and birds have been propagated by AI including giant panda (*Ailuropoda melanoleuca*), cheetah (*Acinonyx jubatus*), black-footed ferret (*Mustela nigripe*), Siberian crane (*Leucogeranus leucogeranus*) and Houbara bustard (*Chlamydotis undulata*) (Ballou 1984, Pukazhenthi & Wildt 2004, Andrabi & Maxwell 2007, Morrow *et al.* 2009, Herrick 2019, Penfold *et al.* 2021). In addition, it is important to highlight the successful utilisation of ART to coral species. To date, there are ~30 species of coral that have been cryobanked from coral reef populations around the world (Hagedorn *et al.* 2019). The frozen-thawed sperm has been used to fertilise eggs from the same spawn, successive spawns, and used for trans-regional IVF of corals (Hagedorn *et al.* 2012, 2017, 2018) separated by hundreds of miles, thereby increasing the heterozygosity of species. The integration of these technologies has helped mitigate the loss of heterozygosity from coral species and continued to aid in global coral conservation efforts (Hagedorn *et al.* 2019).

However, difficulties arise from the huge diversity of both reproductive physiology and behaviour between species of the same taxa, for example, among canids, domestic dogs (*Canis familiaris*) show spontaneous ovulation, whereas the island fox (*Urocyon littoralis*) only ovulates in the presence of a male (Asa *et al.* 2007). Furthermore, in the African wild dog (*Lycaon pictus*), dominant female behaviour regulates reproductive success to alpha females only (Van den Berghe *et al.* 2012). The complications of using techniques developed in the domestic industry for endangered species have resulted in birth rates that are significantly lower than those seen in domestic animals (Mastromonaco & Songsasen 2020). However, there are successful case studies including the now stable endangered black-footed ferret (*M. nigripe*) population (Santymire 2016). The lack of widespread application of ART across all taxa after more than 30 year of efforts highlights the need for alternative approaches. This includes gamete and somatic cell cryopreservation for genome resource banking (Mastromonaco & Songsasen 2020), and the application of aART, even if the production of offspring is many years away. There is also an increasing importance of developing species-specific protocols for ART/aART for endangered species to improve reproductive success rates in the future (Herrick *et al.* 2019, Mastromonaco & Songsasen 2020).

Both ART and aART may raise ethical issues which are rarely explored. The use of aART can remove the invasive manipulation of living animals as these techniques mainly use tissue from neutered or deceased individuals at the point of collection. However, many techniques require invasive manipulation as an end point, for example, surrogacy, cross fostering or tissue implantation and subsequent harvesting of gametes. It is important to note that these procedures must always be performed under general anaesthesia with included analgesia by a highly trained professional, minimising risk to the individuals

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Box 1 Case study preserving amphibians

Amphibians are arguably the class most at risk of extinction (Bishop *et al.* 2012, Ficetola *et al.* 2015) with populations declining faster than any other vertebrate class (Ceballos *et al.* 2015, IUCN SSC Amphibian Specialist Group 2017, Zimkus *et al.* 2018). Significantly challenged by chytridiomycosis (Van Rooij *et al.* 2015), climate change, declining resources, pollution, etc., (Cheng *et al.* 2011), many amphibians including the mountain chicken frog (Leptodactylus fallax) are on the brink of extinction (IUCN SSC Amphibian Specialist Group 2017, Fig. 1A). Amphibians also suffer from reduced research, investment and conservation advancement, including ARTs and aARTs (Kouba *et al.* 2013, Strand *et al.* 2020).

Amphibian IVF has been available since the 1950s; however, this technology has predominantly been used for non-conservation-based research (Clulow *et al.* 2019*a*,*b*). As amphibians utilise external fertilisation, IVF is relatively straight forward compared to that of mammals (Silla *et al.* 2021). After primary publication of frog IVF by Wolf and Hedrick in 1971, little additional research has been conducted (Silla & Byrne 2019). Gamete release can be induced by activation of the hypothalamic–pituitary–gonadal axis (Peter *et al.* 1988, Uteshev *et al.* 2015, Silla & Byrne 2021), and Waggener and Carroll (1998) demonstrated the first example of induced gamete release in Paraguay horned frogs (Lepidobatrachus species) with resultant fertilisation in vitro, thus validating the application of IVF to amphibian conservation. Since then, amphibian IVF has been conducted in several threatened species including Wyoming toad (Bufo baxteri) (Browne *et al.* 2006), corroboree frog (Pseudophryne corroboree) (Byrne & Silla 2010) and dusty gopher frog (Rana sevosa) (Kouba *et al.* 2012).

Amphibian semen can be refrigerated (4°C) for temporary holding or cryopreserved (−196°C) for long-term storage (Browne *et al.* 2002, 2019). For some species, semen has been held at 4°C for 30 days with retained viability (Browne *et al.* 2001), and refrigerated semen has been used in over 40 amphibian species, with outcomes including retrieval of motile sperm and fertilisation in vitro (Browne *et al.* 2001, Keogh *et al.* 2017, Gillis *et al.* 2021*a*). Refrigerated and cryopreserved semen are the two most successful ARTs for amphibians, with semen capable of storage in whole testes and sectioned testicular strips, as well as spermic urine (Poo & Hinkson 2019).

For semen cryopreservation, amphibian spermatozoa appear to be highly tolerant of prolonged exposure to cryoprotectants that other species' cells rarely are (Clulow *et al.* 2019*a*,*b*). However, while semen cryopreservation is generally successful, the theoretical understanding of why the methods work is lacking (Clulow & Clulow 2016). The ability to successfully cryopreserve amphibian oocytes would be a ground-breaking development for conservation (Lawson *et al.* 2013); however, the success of oocyte freezing is low due to the high yolk content and large diameter (Guenther *et al.* 2006). An alternative would be the cryopreservation of embryos; more success may be expected here as early embryonic cells are typically smaller and contain less liquid (Lawson *et al.* 2013).

Cryopreservation of amphibian somatic tissue for use in aART provides additional and vital conservation resources (Strand *et al.* 2020). To date, there has been little research into developing tissue preservation procedures for amphibians (Stand 2021). However, the San Diego Zoo Institute for Conservation Research Frozen Zoo® already holds a large collection of cryopreserved amphibian tissue and cell lines (Chemnick *et al.* 2009), and the IUCN amphibian specialist group have biobank and ART working groups, so advances are being made in this area. One main challenge with amphibian skin cryopreservation and cell culture is contamination from bacteria and fungi (Strauß *et al.* 2013). Strand *et al.* (2021) have shown that even with extensive washing, contamination can still be problematic, especially as liquid nitrogen is known to not fully inhibit the replication of microorganisms (Bajerski *et al.* 2020). Though much work is yet to be done, amphibian cryobanking, ART and aART are undeniably exciting and hold great promise as conservation safety nets.

involved. Nevertheless, ethical and welfare risk assessment should be mandatory prior to the use of them, especially as the welfare of an individual animal risks becoming a secondary consideration after the larger goal of saving a species (de Mori *et al.* 2021). In addition, it could be thought that the time required for successful sample collection, cryopreservation, thawing and use to make viable offspring, with all the research and development involved for species-specific optimisation, could be better spent on more traditional conservation methods (de Mori *et al.* 2021). But, with so many species being lost today, cryopreservation followed by viable cell and tissue cryobanking can fill the gap between permanently losing genetic diversity and the development of future technologies. This review will discuss the potential of cryobanking and the use of reproductive technologies to resurrect biodiversity.

Global prioritisation of species for cryopreservation

Only one aspect of biodiversity conservation, cryobanking, can make significant contributions to population management and species recovery, as seen in the black-footed ferret (*M. nigripes*) and giant panda (*A. melanoleuca*) (Howard *et al.* 2016, Santymire 2016, Comizzoli 2020). However, the immense resources required to sample, maintain and utilise biobanked samples, combined with the sheer number of threatened species requiring conservation intervention globally, mean that not every species can be sampled and conserved in this way (Hobbs *et al.* 2019). The current approach to the selection of species for cryobanking has been mainly opportunistic, with the collection of tissue samples on an *ad hoc* basis, resulting in the prioritisation of large charismatic species and missed

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Figure 1 Critically endangered mountain chicken frog (*Leptodactylus fallax*), photo © Chester Zoo, 2022; photo shared with permission. Chytridiomycosis, volcanic eruptions and habitat loss have resulted in a catastrophic decline in mountain chicken frog numbers, with less than 150 mature individuals now surviving (IUCN SSC Amphibian Specialist Group 2017). Fortunately, somatic tissue samples have been cryopreserved in living biobanks, and with poor *ex situ* breeding success, aART may be an additional conservation tool to prevent this species from going extinct.

conservation opportunities for others (Hobbs *et al.* 2019). If cryobanking is to be an effective and efficient biodiversity conservation tool, then it is important that the way in which we select and prioritise species for storage follows a clear, coordinated and transparent methodology (CPSG 2016, Mooney 2021).

There are multiple ways to integrate cryobanking with wildlife conservation: to support captive breeding programmes and/or to support *in situ* breeding programmes. Each country is likely to have its own set of priorities, and while some might favour the support of threatened populations *in situ*, others will focus on the support of species in *ex situ* populations. *Ex situ* genome banking will likely involve the international transport of cells, tissues and gametes and cryopreservation in facilities outside the home ranges of species. This poses some practical problems, including the risks of disease transmission via the stored samples and via the liquid nitrogen. However, even though the risk of contamination of samples preserved in liquid nitrogen (and subsequent disease transmission) is highly unlikely when appropriate techniques and safe practices are implemented (Penfold *et al.* 2021), it is not possible to move ungulate gametes or embryos in the United States due to the inherent disease transmission risk to the agricultural industry and associated economic threats (Joaquim *et al.* 2017). It is therefore important to guard against this potential disease transmission. Cryobanks set up to serve local species do not run the same risks, although the avoidance of bacterial and viral contamination is still important (Penfold & O'Brien 2012). There are a number of ways to mitigate the risk of disease transmission when transporting genetic material including disease screening of donor animals, high levels of biosecurity and using fresh, previously unused liquid nitrogen (Penfold *et al.* 2021). In the United States, disease transmission risk to the agricultural industry is lower for carnivores, and therefore, transportation has been achieved, for example, embryo transportation of the Brazilian ocelot (*Leopardus pardalis mitis*) (Conforti *et al.* 2009). In Europe, prior to transporation, samples from certain species may require CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) permits. However, within the European Union (EU) generally there is no need for CITES export or import permits. Outside the EU, CITES export permits are required unless the receiving biobank is a registered scientific institution with a CITES exemption.

The utilisation of existing conservation assessment schemes, such as the International Union for the Conservation of Nature (IUCN) Red List, has been suggested as one way to prioritise species for cryobanking efforts, with more threatened species receiving greater priority (Ryder & Onuma 2018). Similarly, considering multiple assessment schemes simultaneously (such as the EDGE (Evolutionarily Distinct and Globally Endangered) of Existence and Alliance for Zero Extinction) can help to identify the most at-risk and uniquely vulnerable species and provide more nuanced species recommendations and prioritisations (Mooney 2021). Prioritising and sampling species on the brink of extinction are invaluable for scientific studies, last-gasp conservation efforts and for any future de-extinction attempts, as seen in the Pyrenean ibex (*Capra pyrenaica pyrenaica*) (Folch *et al.* 2009). However, this also results in the selection of species which already lack genetic diversity within their populations and therefore have limited prospects for meaningful conservation intervention and recovery, ultimately resulting in a limited conservation value of cryobanking such species (Hobbs *et al.* 2019). However, it is possible that gene editing techniques, such as CRISPR-Cas9 (Doudna & Charpentier 2014), may help overcome these problems in the future; but, many ethical considerations will need to be observed (Johnson *et al.* 2016, Segelbacher *et al.* 2021).

To improve the chances of success for conservation intervention and population management, sample collection should focus on species which still have sufficient extant population sizes and genetic diversity available to sample from (Hobbs *et al.* 2019, Ryder & Onuma 2018). This involves a better understanding of species population sizes and genetic diversity and the use of existing assessment

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schemes, for example, the IUCN Red List, to investigate which currently non-threatened species might become threatened in the future and then prioritise these species for cryobanking efforts before they suffer population declines and genetic diversity loss. Prioritising and sampling species while genetic diversity still exists, and before they become threatened, means that early intervention and genetic restoration are possible once their populations begin to decline, improving the probability of successful species recovery (Hobbs *et al.* 2019). Although predicting which species will become threatened, and why, is difficult (Walker *et al.* 2021), studies such as that by Foden *et al.* (2013) have identified species which are most vulnerable to future climate change, even those not currently threatened with extinction, such as the griffon vulture (*Gyps fulvus*), and can provide potential new priorities for conservation and cryobanking efforts (Mooney 2021).

Similarly, opportunities for sample collection should be incorporated into the species prioritisation process, as many species are found in isolated or inaccessible locations, making sample acquisition and transport both difficult and expensive (Ryder & Onuma 2018, Houck 2019). However, the global zoo and aquarium community represent a unique resource for samples, either through existing collections such as, for example, the European Association of Zoos and Aquaria (EAZA) Biobank, or new sampling drives, and can provide easier access to populations of thousands of species which are currently threatened or likely to become threatened in the future (Mooney 2021). Additionally, many zoos and aquariums have active links and partnerships with *in situ* conservation projects, providing opportunities to collect additional genetic samples from already represented species and from species which are not currently maintained *ex situ* (AZA 2015). By capitalising on sampling opportunities and utilising *ex situ* collections and their partnerships, we can reduce sampling costs and increase the probability that biobanked samples can be employed to help conserve and manage both *in situ* and *ex situ* populations in the future (Benirschke 1984, Clarke 2009, Mooney 2021).

The process of prioritising *ex situ* managed species for cryobanking also needs to consider which individuals within the population are the most genetically valuable and suitable for future conservation efforts, maximising the conservation value of banked samples and limiting the loss of genetic diversity within a population (Clarke 2009). Many of the species found in zoos and aquariums are being actively managed to maintain genetic diversity through regional or international population management programmes (Che-Castaldo *et al.* 2021), and the availability of such pedigree managed and potentially also genotyped populations can help to identify the most genetically valuable individuals to sample. Such strategic cryobanking efforts have helped to reintroduce once lost genetic variation into extant populations of black-footed ferrets (*M. nigripes*), using samples collected in 1988 from an individual which had no living descendants and was no longer genetically represented in the population (Imbler 2021) and the endangered Przewalski's horse (*Equus przewalskii*) which was cloned in 2020 using samples cryopreserved in 1980 at the San Diego Zoo Institute for Conservation Research Frozen Zoo®. Unfortunately, for the many species which have yet to be sampled, such opportunities are not available, limiting the options open to conservation practitioners and population managers. Cryobanking needs to be seen as an integral part of the conservation toolkit and when used appropriately can even reduce the costs required to achieve genetic diversity retention targets compared to traditional *ex situ* breeding strategies (Howell *et al.* 2021). However, this will require the combining of both species and individual animal prioritisations to provide the most effective use of bio and cryobanking as conservation and population management tools.

Gamete and reproductive tissue cryopreservation

Spermatozoa cryopreservation is an example of just one ART that can facilitate a living cryobank capable of aiding in the genetic management of endangered species and has been achieved in many species (covered in-depth elsewhere, for example, amphibians: Browne *et al.* 2019; fish: Asturiano *et al.* 2017, Xin *et al.* 2017; mammals: Swanson *et al.* 2007, Rickard *et al.* 2022; avians: Asano & Tajima 2017, Cardoso *et al.* 2020). Storing genes in the form of spermatozoa is particularly beneficial as spermatozoa are continuously replenished haploid cells that can be collected from numerous genetically diverse representatives of a species. Furthermore, the cryopreservation of genes in the form of spermatozoa enables the application of ART to reintroduce genes back into populations. ART techniques such as AI, IVF, embryo transfer or intra cytoplasmic sperm injection (IVF, ET, ICSI) are currently the most efficient treatment modalities practised compared to alternative aART methods such as somatic nuclear transfer (Choudhary *et al.* 2016, Gouveia *et al.* 2020), with AI currently remaining the most efficient and commonly used ART technique (Holt & Lloyd 2009).

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Semen can be collected from non-domestic species in many ways which include the use of an artificial vagina, transrectal massage (Schmitt & Hildebrandt 1998), electroejaculation (Roth *et al.* 1998), testicular sperm aspiration (Damiani *et al.* 2004), urethral catheterisation (Lueders *et al.* 2012) or post-castration dissection (ante-mortem or post-mortem) (Saragusty *et al.* 2010, Roth *et al.* 2016). The process of collecting and freezing spermatozoa from testes post-mortem is commonly referred to as 'gamete rescue' and is used by scientists to prevent the permanent loss of a male genetics from a population. Once spermatozoa are collected, they are diluted in a cryopreservation medium, which is formulated to mitigate damage inflicted by the cryopreservation process (Purdy 2006, Comizzoli *et al.* 2012). In general, cryopreservation mediums contain (1) energy substrates for spermatozoa to metabolise; (2) antioxidants to prevent the build-up of reactive oxygen species; (3) buffers to prevent harmful shifts in pH; (4) osmolytes to create an isosmotic solution; (5) plant or animal source proteins and/or lipids to stabilise the membrane; (6) antibiotics to mitigate potential risks of bacterial disease transmission and (7) a cryoprotectant (such as glycerol, DMSO, ethylene glycol, etc.), which slow down the kinetics of ice crystal formation, preventing the formation of lethal intracellular ice (Holt 2000, Fuller 2004). As each species' physiology is inherently unique, cryopreservation mediums must be formulated to meet species-specific physiologic requirements and mitigate that species' sensitivities to cryopreservation (Comizzoli *et al.* 2012).

Similar to cryopreservation media, methods to cryopreserve spermatozoa are highly diverse across taxa. As a sample is cooled and ice begins to form, the remaining solution becomes increasingly concentrated. The increased concentration of the solution results in the dehydration of the spermatozoa, preventing the formation of lethal intracellular ice. However, the increased concentration of the solution can also elicit toxic effects if the spermatozoa are exposed to the solution for too long. An optimal cooling rate varies between species and cell type, but in general, an optimal cooling rate is achieved when the rate is slow enough that spermatozoa can be dehydrated, preventing the formation of intracellular ice, but fast enough that the spermatozoa are not exposed to changes in the solution for too long. Once cryopreserved, viable spermatozoa can be stored almost indefinitely without decomposition or metabolism, typically beneath liquid nitrogen, in 'suspended animation' until thawing.

Even though there are some positive examples, like the black-footed ferret (*M. nigripes*, Howard *et al.* 2016) and certain coral species (Hagedorn *et al.* 2012, 2017), successful production of live offspring using frozen-thawed spermatozoa can be extremely variable and challenging for different species (Leibo & Songsasen 2002, Comizzoli *et al.* 2015). This can be observed most notably in marsupials (Taggart *et al.* 1996, Unwin & Pettit 2004). So far, it has not been possible to cryopreserve any marsupial spermatozoa successfully and the only successful artificial insemination in a marsupial was achieved in the koala (*Phascolarctos cinereus*) using chilled, but not frozen, spermatozoa (Johnston & Holt 2019). In this example, it is thought that dilution of koala semen for artificial insemination is complicated because koalas are induced ovulators, and it is thought that ovulating factors are present in the semen. Therefore, the extension of semen for preservation purposes, which involves significant dilution, might be anticipated to result in a failure to induce ovulation (Allen *et al.* 2008).

In other species where cryopreservation has been attempted but failed, it is likely that the failures are also due to complex underlying factors affecting viability or fertilising ability that are still poorly understood or, yet, unknown by researchers. These include factors relating to species that are relatively important from both commercial and conservation perspectives such as swine (Bailey *et al.* 2000, 2008), avian (Blesbois *et al.* 2005) and elasmobranch (Gillis *et al.* 2021*b*).

Establishing banks of frozen and viable semen from such species using conventional freezing methods is therefore not possible at present. However, it has been proposed that, on balance, spermatozoa is still worth freezing in the hope that techniques that can take advantage of the genetic material contained in currently non-viable cryopreserved gametes will become available sometime in the future (Rodger *et al.* 2019). Being able to quickly develop an optimal spermatozoa cryopreservation protocol for the variety of species selected as suitable candidates for cryobanking, and standardisation of these protocols, is the main challenge for researchers, especially when faced with extremely limited biological material to effectively develop a working protocol when an opportunity for spermatozoa collection arises. Furthermore, there are two important phenomenon that should also be considered. First, inbreeding depression can lead to poor spermatozoa morphology, resulting in poor fertility (Huffmeyer *et al.* 2022). Secondly, wild species subject to lower levels of spermatozoa competition (for instance, those limited by population size) may also result in an increase in the variability of spermatozoa morphology (Carballo *et al.* 2019). A morphologically homologous sperm

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population is a prerequisite for developing an optimal cryopreservation protocol; therefore, factors that lead to variability in morphology will significantly impede the likelihood of developing a successful protocol. However, more research is needed to ascertain the degree to which inbreeding depression and spermatozoa competition leads to poor semen freezing ability, and the precise evolutionary mechanism is yet to be explored.

Spermatozoa is commonly cryopreserved from domestic species for commercial breeding programmes with high levels of success, including domestic species of cattle, water buffalo, cats, rodents, horse, goat, deer, sheep, dog, rabbit and selected fish species (Curry 2000, Woelders *et al.* 2012, Kochan *et al.* 2019, Thongphakdee *et al.* 2020). It is prudent to utilise spermatozoa cryopreservation protocols already established in well-developed specimens as a reliable model for poorly understood specimens, including for species from the same genera or closely related species (Comizzoli 2015). This has been successfully demonstrated with numerous critically endangered species already, including the use of equine semen protocols as a model for rhinoceros species (Reid *et al.* 2009), bovine semen protocols as a model for gazelle species (Saragusty *et al.* 2006), domestic ferret as a model for black-footed ferret (*M. nigripes*) (Howard *et al.* 2016) and human spermatozoa cryopreservation protocols as a model for macaques (Si *et al.* 2010). However, for some, closely related species to use as semen cryopreservation models, do not exist. Protocols have been successfully using already established methods with slight modifications to take the variability in semen characteristics into account. Examples where this can be observed include Asian elephants (*Elephas maximus*) (Saragusty *et al.* 2009), giant panda (*A. melanoleuc*) (Martin-Wintle *et al.* 2019), bees (Comizzoli *et al.* 2019), killer whale (*Orcinus orca*) and bottlenose dolphin (*Tursiops truncatus*) (O'Brien & Robeck 2006, Robeck *et al.* 2011). A thorough understanding of the phylogenetic relationship of species is therefore important when planning an effective strategy for cryopreserving sperm from novel species. Developing forums that actively encourage knowledge transfer between cryobiologists, adequate data capture and sharing of proven spermatozoa cryopreservation protocols are also mission critical to creating an effective living biobank. Further research is also required to better understand the root causes why some species produce more cryo-sensitive spermatozoa than others. Innovative work in this field includes the successful application of novel ART such as control-rate freezers, directional cryopreservation (Saragusty *et al.* 2007, Reid *et al.* 2009), vitrification (Hunt 2017) and freeze drying of spermatozoa (Sherman 1963, Kaneko *et al.* 2014).

In addition, spermatozoa can be collected from the epididymis of testes following death or neutering of sexually mature individuals. However, if this technique fails, or the individual is immature, the testis remains a viable source of spermatozoa (Crabbé *et al.* 1997). Indeed, in domestic cats, a model for endangered wild felid species, spermatozoa has been successfully removed from cryopreserved testicular tissue by mincing thawed tissue. Via ICSI, embryos were then created, resulting in live kittens (Tharasanit *et al.* 2012). Furthermore, testicular spermatozoa has the potential of retaining higher viability (Chatdarong 2011). The cryopreservation of testicular tissue also increases the potential of saving important genetics from valuable animals that died unexpectedly. There are multiple techniques for the preservation of testicular tissue. Following cryopreservation, testicular fragments can be cultured *in vitro* to obtain viable spermatozoa, and techniques including ultra-rapid freezing have resulted in promising results (Sato *et al.* 2011). Effective preservation of testes is vital to maintain the functionality of retrieved spermatozoa (Pothana *et al.* 2017, da Silva *et al.* 2020). The cryopreservation of testicular tissue has been achieved for many wild mammalian species (Table 1), although this is more complex than cell cryopreservation due to increased requirements of permeation of cryoprotectant and increased heterogeneity of the tissue (Pothana *et al.* 2017).

Slow freezing methods for testicular tissue have shown promising results with tissue showing maintenance of spermiogenesis after cryopreservation for nonhuman primates including white-headed marmoset (*Calllithrix geoffroyi*), mandrill (*Mandrillus sphinx*) and chimpanzee (*Pan troglodytes*) (Pothana *et al.* 2016). Following cryopreservation, conditions need to be met to enable the thawed tissue to resume spermatogenesis. One option is the autologous grafting of the thawed tissue (autografting: grafting tissue from original location to elsewhere in the same individual). This has been achieved for rhesus macaques (*Macaca mulatta*) where grafted testicular tissue produced spermatozoa, which was retrieved and used to fertilise oocytes by ICSI, resulting in embryos and a successful graft-derived baby (Fayomi *et al.* 2019). While complete spermatogenesis after testicular tissue cryopreservation and xenografting (grafting tissue from a donor animal into a recipient of another species) has not been achieved for adults of wild or domestic species, it has been achieved for immature individuals of ovine and swine (Arregui *et al.* 2008, Silva *et al.* 2020). However, autografting and xenografting of testicular tissue have little application for endangered species (Silva *et al.* 2020). An alternative method is *in vitro* culture of testicular tissue to

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Table 1 Species for which cryopreservation of testicular tissue has been achieved.

Species	Reference
Primates	Poels *et al.* (2012), Pothana *et al.* (2016), Fayomi *et al.* (2019)
Rhesus monkey (*Macaca mulatta*)	
Mandril (*Mandrillus sphinx*)	
Chimpanzee (*Pan troglodytes*)	
White-headed marmoset (*Callithrix geoffroyi*)	
Cervids	Thuwanut *et al.* (2013), Pothana *et al.* (2015, 2017)
Indian spotted mouse deer (*Moschiola indica*)	
Indian hog deer (*Hyelaphus porcinus*)	
Barking deer (*Muntiacys muntjak*)	
Sambar deer (*Rusa univolor*)	
Rusa deer (*Rusa timorensis*)	
Fea's muntjac (*Muntiacus feae*)	
Bovids	Thuwanut *et al.* (2013)
Sumatran serow (*Caprivornis sunatraensis*)	
Felids	Thuwanut *et al.* (2013)
Jungle cat (*Felis chaus*)	
Lion (*Panthera leo*)	
Leopard (*Panthera pardus*)	
Canids	Andrae *et al.* (2021)
Grey wolf (*Canis lupus*)	
Suids	da Silva et al. (2019)
Collard peccary (*Dicotyles tajacu*)	

initiate spermatogenesis (Lee *et al.* 2013, Richer *et al.* 2020). The success of *in vitro* testicular tissue culture is reliant on specific methodologies which are still to be established for endangered species making it vital to cryopreserve and biobank this tissue (Lima *et al.* 2020). Biobanking of tissue maintains genetic variability across time and space providing the opportunity to first develop and optimise the necessary technologies (Hildebrandt *et al.* 2021).

Mature oocytes can be harvested from ovarian follicles, and immature oocytes can be collected from ovarian tissues. Due to the low surface area-to-volume ratio of the oocyte, increased levels of intracellular ice formation during freezing makes cryopreservation more challenging; however, it has been attempted in a number of species (Table 2) (Borini & Bianchi 2012). This damage during the cryopreservation process is exacerbated by the low and variable membrane permeability to cryoprotectants (dependent on oocyte development state) (Leibo 1980, Arav 2014, García-Martínez *et al.* 2021), resulting in cellular disruption and death and leading to generally poor fertilisation rates from frozen-thawed oocytes (Tharasanit & Thuwanut 2021). Furthermore, with reference to cryopreserving oocytes from endangered animal species, the oocyte membrane permeability to cryoprotectant agents varies among species, again leading to theoretical models being used to predict likely optimal freezing protocols (Tharasanit & Thuwanut 2021). Oocyte cryopreservation is particularly challenging in fish due to the large cell volume, multiple compartments, the presence of a chorion, the low membrane permeability to cryoprotectants and a high chilling sensitivity (Asturiano *et al.* 2017, Diwan *et al.* 2020). Therefore, for those species, alternatives are intensively investigated and germ cell surrogacy via germ cell transplantation looks like one of the most promising methods (Rivers *et al.* 2020). Technical difficulties confronted during fish oocyte cryopreservation were already ominous for amphibian oocyte handling. The same large diameters, volumes and high yolk content can be observed in both taxa and are barriers in efficiently applying cryopreservation methods (Clulow *et al.* 2019*a*,*b*) (also see case study Fig. 1). In some species, such as the domestic cat, oocytes contain high levels of lipid droplets that become disrupted during slow freezing procedures resulting in cellular injury (Okotrub *et al.* 2018). This may well apply to endangered felines too. As an alternative, vitrification, which avoids ice formation by using high concentrations of cryoprotectants and very rapid freezing, resulting in solidification without ice formation (Rall & Fahy 1985), has been successfully employed in oocyte cryopreservation, and, despite some problems (Prentice & Anzar 2010), the evidence suggests that this is the preferred method, at least for some species (Rienzi *et al.* 2017, Whaley *et al.* 2021). Cryopreservation of feline oocytes, domestic and non-domestic species, however, remains in an experimental phase (Jewgenow & Zahmel 2020). Post-thawing viability and developmental competence are seriously impaired and until now, none of the existing techniques could significantly improve

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Table 2 Examples of mammalian species for which oocyte cryopreservation has been conducted.

Species	Method	Reference
Bovine	Vitrification	Fuku *et al.* (1992), Hamano *et al.* (1992), Hurtt *et al.* (2000), Chian *et al.* (2004), Vieira *et al.* (2008), Nakayama *et al.* (2020)
Equine	Slow freezing	Otoi *et al.* (1995), Suzuki *et al.* (1996)
	Vitrification	Hurtt *et al.* (2000), Maclellan *et al.* (2002), Ortiz-Escribano *et al.* (2018), Clérico *et al.* (2021)
Ovine/caprine	Slow freezing	Bhat *et al.* (2014)
	Vitrification	Purohit *et al.* (2012), Moawad *et al.* (2013), Bhat *et al.* (2014), Quan *et al.* (2016)
Porcine	Slow freezing	Yang *et al.* (2012)
	Vitrification	Vallorani *et al.* (2012), Appeltant *et al.* (2017), Jia *et al.* (2019), López *et al.* (2021)
Canine		
Domestic	Vitrification	Abe *et al.* (2010), Turathum *et al.* (2010)
Mexican grey wolf (*Canis lupus baileyi*)	Vitrification	Boutelle *et al.* (2011)
Blue fox (*Alopex lagopus*) farmed	Vitrification	Zhou *et al.* (2009)
Feline		
Domestic	Slow freezing	Luvoni and Pellizzari (2000)
	Vitrification	Fernandez-Gonzalez and Jewgenow (2017), Nowak *et al.* (2020), Sowińska *et al.* (2020), Fernandez-Gonzalez *et al.* (2021)
Non-human primate		
Lowland gorilla (*Gorilla gorilla gorilla*)	Slow freezing	Lanzendorf *et al.* (1992)
Macaque (*Macaca mulatta*)	Slow and rapid freezing	Vandevoort *et al.* (2008)

freezing (Jewgenow & Zahmel 2020). Since there is scarcity in wild feline samples, ART protocols are being developed in the domestic cat and seem to be working out well as a model for the wild species (Fernandez-Gonzalez *et al.* 2021) although more research is required to verify gamete rescue methods in exotic felids (Jewgenow & Zahmel 2020).

An alternative approach to freezing oocytes is the cryopreservation of immature-oocyte-containing ovarian tissue, which has been conducted in a number of species (Table 3) (Martinez 2017). Since the first mouse was born in 1996 following *in vitro* growth of primordial follicles (Eppig & O'Brien 1996), there have been many published studies on this technique in a number of species including white-tailed deer (*Odocoileus virginianu*) (Gastal *et al.* 2018), domestic cat (*Felis catus*) (Mouttham & Comizzoli 2016), collared peccary (*Pecari tajacu*) (Lima *et al.* 2019), yellow-toothed cavies (*Galea musteloides*) (Praxedes *et al.* 2017), brown trout (*Salmo trutta*) (Lujić *et al.* 2017), donkey (*Equus asinus*) (Lopes *et al.* 2018) and domestic cattle (Figueiredo *et al.* 1993, 1994*a*,*b*, Hulshof *et al.* 1995, Vasconcelos *et al.* 2013). Following death, euthanasia or neutering, a portion of the ovary can be cryopreserved; the immature follicle-containing cortex is dissected and cut into small strips under sterile conditions before slow freezing (Benesova & Trefil 2016, Hinkle *et al.* 2021). As the ovary contains many follicles, there is a potential to produce large numbers of oocytes from the tissue within a laboratory. Harvested oocytes can be matured *in vitro* and used in IVF. Teams, including the Rhino Fertility Project, are developing the *in vitro* tissue culture technique to safeguard critically endangered species, including the northern white rhino (*C. simum cottoni*), of which there are only two individuals left, again highlighting the critical importance of viable cell cryobanking for resurrecting biodiversity. Several initiatives such as the Hemmersbach Rhino Force Cryovault (South Africa), Rhino Repro (South Africa), the Frozen Zoo (San Diego Zoo Wildlife Alliance, USA) and BioRescue (Germany) are storing rhinoceros tissue and genetic material that can be utilised once methods to produce rhinoceros calves *in vitro* will be established.

Somatic cell cryopreservation and advanced assisted reproductive technology

Reproductive cloning involves the transfer of genetic material from a somatic cell into an enucleated oocyte, SCNT, ultimately resulting in an animal that has a genome sequence within the nucleus identical to that of the donor of the somatic cell used. The ability of differentiated adult cells to produce viable offspring following SCNT was first demonstrated in the African clawed frog (*Xenopus*

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Table 3 Examples of ovarian tissue cryopreservation: domestic/laboratory and wild animal species.

Species	Freezing methodology	Cryoprotectants	Outcome	Reference
Domestic/laboratory				
Murine	Vitrification	Ethylene glycol and DMSO	Melatonin improved outcome post-thaw	Wu *et al.* (2019)
Porcine	Vitrification	Ethylene glycol		Jia *et al.* (2020)
Canine	Slow freezing	DMSO and propanediol	DMSO more effective as a cryoprotectant	Lopes *et al.* (2016)
Feline	Vitrification	DMSO and ethylene glycol	Use of metal (titanium) freezing tubes proved advantageous	Fernandez-Gonzalez *et al.* (2021)
Caprine	Slow freezing	DMSO and propanediol		Rodrigues *et al.* (2004)
Ovine	Slow freezing Vitrification	DMSO and sucrose DMSO, ethylene glycol and sucrose	No significant differences between techniques	Locatelli *et al.* (2019)
Bovine	Vitrification	DMSO and ethylene glycol	Leucosporidium ice-binding protein reduced post-thaw damage	Kong *et al.* (2021)
Wild				
Agouti (*Dasyprocta*)	Slow freezing	DMSO, ethylene glycol and propanediol		Wanderleya *et al.* (2012)
African lion (*Panthera leo*)	Slow freezing	Ethylene glycol and sucrose		Wiedemann *et al.* (2012)
Zebu (*Bos indicus*)	Slow freezing	Glycerol, DMSO, ethylene glycol and propanediol	DMSO and propanediol were the most effective cryoprotectants	Lucci *et al.* (2004)
Amur leopard (*Panthera pardus orientalis*), black-footed cat (*Felis nigripes*), Geoffroy's cat (*Leopardus geoffroyi*), northern Chinese leopard (*Panthera pardus japonensis*), oncilla (*Leopardus tigrinus*), serval (*Lupus cervarius*), sumatran tiger (*Panthera tigris sondaica*)	Slow freezing	Ethylene glycol and sucrose		Wiedemann *et al.* (2013)
Mexican grey wolf (*Canis lupus baileyi*)	Vitrification			Boutelle *et al.* (2011)

laevis) by Gurdon *et al.* (1975) who showed that nuclei from keratinised skin cells transplanted into enucleated oocytes could develop into viable tadpoles, establishing the principle that cell nuclei do not undergo irreversible changes as the cell specialises to form adult tissues. The importance of this fundamental finding has been recognised by the award of the Nobel Prize for Physiology or Medicine jointly to Gurdon in 2012. The successful application of nuclear transfer using adult somatic cells to a mammalian species, the sheep (*Ovis aries*), was reported by Wilmut *et al.* (1997) resulting in the first cloned mammal.

SCNT can be performed either by removing the nucleus from the somatic cell and introducing it into the enucleated oocyte by microinjection (Wakayama *et al.* 1999), or, alternatively, the entire somatic cell can be fused with the enucleated oocyte using electrical fusion (Liu *et al.* 2015), of which, the latter appears to be the preferable approach (Qu *et al.* 2020). Oocyte maturation is required prior to fusion, with the optimum conditions varying between species (Borges & Pereira 2019). Following artificial activation, for example, with ionomycin and 6-dimethylaminopurine, which has been successful in many different species including bovines (Bhak *et al.* 2006), camelids (Wani *et al.* 2017), porcines (Borges *et al.* 2020) and primates (Liu *et al.* 2018), the egg develops to an early embryo *in vitro* and is then implanted into the uterus of suitable recipient female. Identification and optimisation of the three critical procedures with the greatest impact on the

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development of oocytes and early embryos, namely oocyte micromanipulation, electrofusion, and the *in vitro* culture of early embryos, have recently been reviewed (Ma *et al.* 2021).

The possibility of using reproductive cloning in the conservation of endangered animal species has been widely discussed (e.g., Holt *et al.* 2004, Shapiro 2017, Borges & Pereira 2019). For any endangered species, it is unlikely that sufficient oocytes will be available for SCNT. Thus, cells of a separate but related species will need to be used: interspecific SCNT (iSCNT, Fig. 2). If successful, the result is the birth of an animal with the nuclear genome of the endangered individual but with the mitochondrial DNA derived from the donated oocyte. As both nuclear and mitochondrial genes regulate mitochondrial development and function (Mrowiec *et al.* 2021), the more closely related the species of the donor nucleus and recipient enucleated oocyte, the less likely there will be nuclear-mitochondrial incompatibility (Lagutina *et al.* 2013). Species or individuals created by means of interspecific cloning are considered by IUCN as 'proxies' which are the functional equivalents of an extinct species able to restore ecological functions or processes that might have been lost because of the extinction of the original species (IUCN 2016). However, because of, for example, microbiome differences and inheritance of the mtDNA of the donor oocyte, proxies result in a species that differs from the extinct one (IUCN 2016). Besides technical and biological hurdles, legal and ethical considerations need to be taken into account when approaching de-extinction efforts based on proxies (Seddon & King 2019).

Low success rates have been reported for reproductive cloning resulting in only 5–10% of reprogrammed embryos yielding viable offspring, with many factors affecting this success rate (Long *et al.* 2014). These factors include DNA damage, which can be improved by upregulating modulators of the DNA damage response (Lee *et al.* 2021), the cell type used for nuclear donation (Inoue *et al.* 2005, Liu *et al.* 2015, Lee *et al.* 2019) and the mismatch of mitochondrial DNA between donor cell and recipient oocyte (Takeda 2019, Mrowiec *et al.* 2021). Epigenetic processes may also affect DNA replication and transcription (Gouveia *et al.* 2020). Many of these problems occur early on in embryonic development and result from incomplete reprogramming of the donor cell nuclei and the subsequent developmental failure of the cloned embryos (Zuo *et al.* 2014). Choice of oocyte donor species to ensure compatibility with the somatic cell donor is also likely to be an important factor (Jeon *et al.* 2016).

It is particularly important to take the low success rates of reproductive cloning into consideration in the context of endangered species, where the production of viable offspring is the top priority. Although iSCNT has been applied to many species resulting in the birth of offspring, by no means all of these were viable in the long term for several reasons including morphological abnormalities, premature delivery, lung immaturity, stillbirths, placental separation and septicaemia (Table 4). Furthermore, cloning results in offspring genetically identical to the somatic cell donor and needs careful consideration where the gene pool of an endangered species is limited. In the future, it may be possible to use genome editing with CRISPR/Cas9 to address this issue (Sheets *et al.* 2016), albeit it also raises ethical concerns. Cryopreservation of somatic cells taken from as many different tissues as possible from each

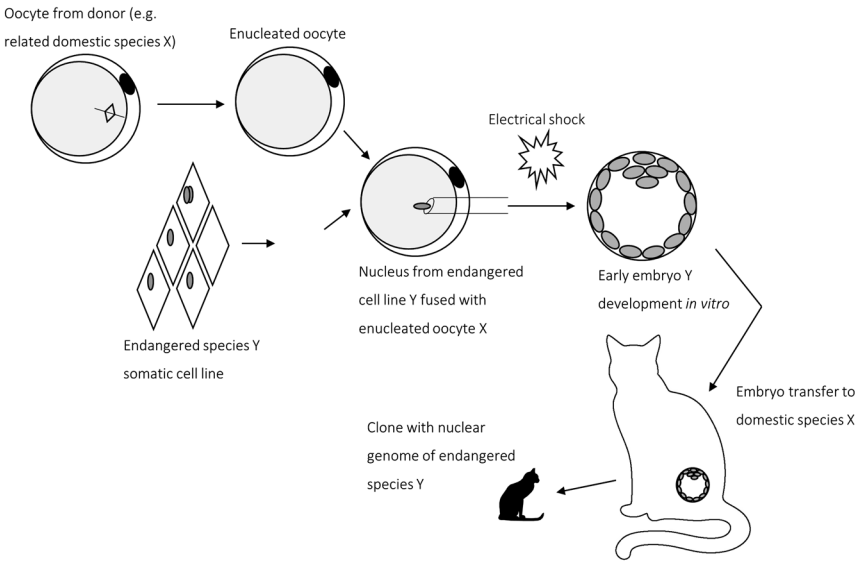

Figure 2 Outline of the interspecific somatic cell nuclear transfer (iSCNT) procedure. The nucleus from an endangered species' somatic cell (species Y) is fused with the enucleated oocyte from a closely related, domestic species (species X). Following electrical or chemical activation, an early embryo of species Y developed *in vitro*. The early embryo is transferred into a surrogate mother of domestic species X, resulting in a clone containing the nuclear genome of endangered species Y.

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Table 4 Examples of interspecific somatic cell nuclear transfer (iSCNT) of mammalian species including oocyte and nuclear donor. The International Union for the Conservation of Nature (IUCN) red list status of the nucleus donor species is also included. Many of the resulting offspring did not show long-term survival, and outcome is noted where available.

Oocyte donor	Nucleus donor	IUCN red list status of nucleus donor	Outcome	Reference
Domestic cat (*Felis catus*)	African wild cat (*Felis silvestris lybica*)	Least concern (subspecies unclear)	17 kittens, 2 survived long term	Gómez *et al.* (2004)
Domestic cat (*F. catus*)	Sand cat (*Felis margarita*)	Least concern	1 of 14 kittens born survived 2 months	Gómez *et al.* (2008)
Domestic cat (*F. catus*)	Cheetah (*Acinonyx jubatus*)	Vulnerable	Incomplete nuclear reprogramming	Moro *et al.* (2015)
Domestic cat (*F. catus*)	Kodkod (*Leopardus guigna*)	Vulnerable	Embryos only developed to the morula stage	Veraguas *et al.* (2020)
Domestic cow (*Bos taurus*)	Banteng (*Bos javanicus*)	Endangered	2 calves, 1 survived long term	Janssen *et al.* (2004)
Domestic sheep (*Ovis aries*)	Mouflon (*Ovis orientalis musimon*)	Near threatened	1 lamb, 'apparently normal'	Loi *et al.* (2001)
Domestic sheep (*O. aries*)	Esfahan mouflon (*Ovis orientalis isphahanica*)	Vulnerable	2 lambs, both died shortly after birth	Hajian *et al.* (2011)
Spanish Ibex (*Capra pyrenaica*)	Pyrenian ibex, Bucardo (*Capra pyrenaica pyrenaica*)	Least concern (subspecies extinct)	1 kid, died shortly after birth	Folch *et al.* (2009)
Dromedary (*Camelus dromadarius*)	Bactrian camel (*Camelus bactrianus*)	Critically endangered	1 calf, died on day 7 post-partum	Wani *et al.* (2017)
Domestic dog (*Canis familiaris*)	Grey wolf (*Canis lupus*)	Least concern	4 pups, 3 survived long term	Oh *et al.* (2008)
Domestic dog (*C. familiaris*)	Coyote (*Canis latrans*)	Least concern	8 pups, all viable	Hwang *et al.* (2012)
Domestic ferret (*Mustela furo*)	Black-footed ferret (*Mustela nigripes*)	Endangered	1 pup, survived long term	Sandler *et al.* (2021)
Macaque monkey (*Macaca mulatta*)	Crab-eating macaque (*Macaca fascicularis*)	Vulnerable	2 young, healthy	Liu *et al.* (2018)

endangered animal should be conducted and stored until significant advances have been made in our understanding of the reproductive biology of individual species. This will maximise the potential of reproductive cloning in the conservation of endangered animal species, for example, the black-footed ferret (*M. nigripes*) and Przewalski's horse (*E. przewalskii*). If we fail to collect and store these tissues now, they are gone forever.

In addition, the cryopreservation of tissues from endangered species, capable of differentiation to germ cells, could provide another way of improving genetic diversity, particularly where only small numbers of individuals remain. Embryonic stem cells can differentiate into all cell types of the body including oocytes (Hübner *et al.* 2003) and spermatozoa (Toyooka *et al.* 2003). As these cells are derived from the early embryo that is destroyed in the process, their use for endangered species is inappropriate. Induced pluripotent stem (iPSCs are derived from adult somatic cells by genetic reprogramming to an embryonic stem cell-like state (Takahashi & Yamanaka 2006) and have been used in attempts to regenerate endangered species (Fig. 3). However, some iPS cell clones differ from embryonic stem cells in several ways including gene expression, DNA methylation and cell differentiation (reviewed by Yamanaka 2012) resulting in a number of potential problems including increased immunogenicity (Okita *et al.* 2011). Such factors need to be taken into account when considering the use of iPS cell technology.

For the cryopreservation and storage of samples from endangered animals, the choice of tissue is an important consideration. The tissue chosen should yield viable cells following freezing and thawing and should be readily reprogrammable to generate iPS cells. The viability of fibroblasts obtained from a wide range of taxa including domestic (e.g. pig (Liu *et al.* 2014), sheep (Na *et al.* 2010) and cow (Li *et al.* 2009) and endangered species (e.g. Bengal tiger (*Panthera tigris tigris*) (Guan *et al.* 2010), brown brocket deer (*Mazama pandora*) (Magalhães *et al.* 2017) and jaguar (*Panthera onca*) (Mestre-Citrinovitz *et al.* 2016) has been demonstrated post freeze/thaw. Along with this demonstrable viability, the ability to successfully reprogramme fibroblasts to iPS cells render this cell type an obvious choice. Indeed, a number of biobanks, including the San Diego Zoo Institute for Conservation Research Frozen Zoo® and The Leibniz Institute for Zoo and Wildlife Research, maintain a collection of frozen

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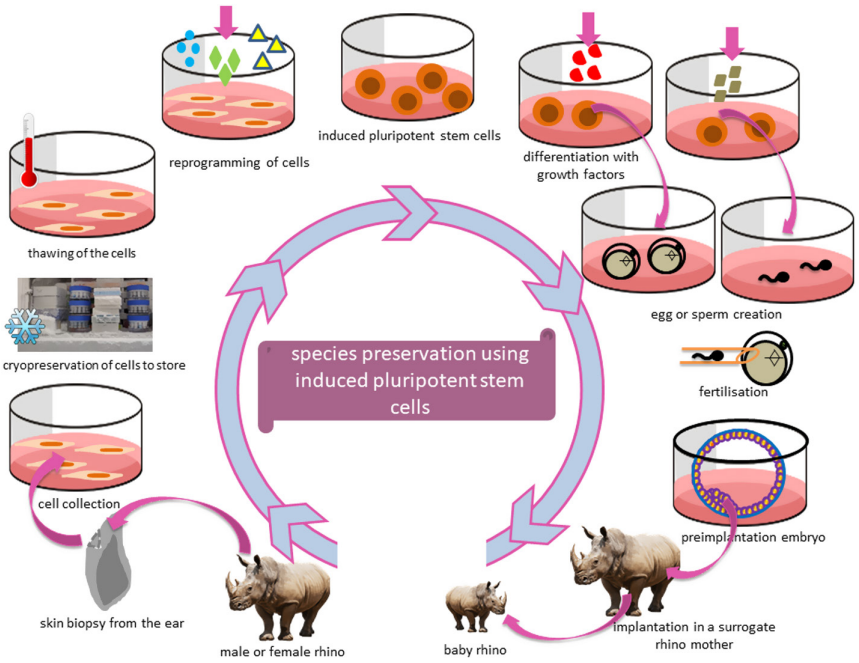

Figure 3 Species preservation using induced pluripotent stem (iPS) cells, which can be differentiated into oocytes or spermatozoa using the rhinoceros as an example. After a biopsy of a recently deceased animal, cells can be cryopreserved until the moment they are needed to produce oocytes or spermatozoa. Those differentiated cells will first need to be reprogrammed to obtain pluripotency. Afterwards, growth factors can re-differentiate the cells into the desired cell population (oocytes or spermatozoa). *In vitro* fertilisation will result in an embryo, which can be transplanted into a surrogate mother leading to offspring.

fibroblasts from multiple endangered species. In the context of cryobanking, iPS cells were first developed from frozen fibroblasts from the endangered primate the drill (*Mandrillus leucophaeus*) and the critically endangered northern white rhinoceros (*C. simum cottoni*) using viral vectors carrying the human sequences of reprogramming factors Oct4, Sox2, cMyc and KLF4 (Ben-Nun *et al.* 2011). Perhaps somewhat surprisingly, the rhinoceros responded to the human reprogramming factor sequences, suggesting that the reprogramming mechanism is highly conserved between different species, which bodes well for the potential application of this approach to a range of species. One caveat is that reprogrammed iPS-like cells from certain species, for example bovines, do not appear to yield sustainable cell lines (Pillai *et al.* 2019). The *in vitro* development of gametes from iPS cells, and the subsequent generation of viable embryos, is key to the application of this technology to the successful prevention of extinction of endangered species. The reconstitution of gametes from pluripotent stem cells, both oocytes (Hikabe *et al.* 2016, Hamazaki *et al.* 2021) and spermatozoa (Li *et al.* 2013, Ishikura *et al.* 2021), has been achieved in the mouse, and primordial germ cells have been developed from iPS cells in the northern white rhinoceros (*C. simum cottoni*) (Korody *et al.* 2021). iPS cells have now been derived from a variety of species (Table 5).

Before iPS cells or even SCNT techniques being used to produce viable offspring, a much greater knowledge of the reproductive biology of both the embryo and the surrogate dam will be vital. Pregnancy is a major challenge for the mammalian maternal immune system with specific mechanisms including the induction by the decidua of regulatory M2 macrophages and Treg cells to elicit immune tolerance at the foetal–maternal interface to prevent rejection of the semi-allogeneic (sharing only half the genes of the mother) foetus (Lindau *et al.* 2021). The increased rate of spontaneous abortion seen in cattle pregnancies produced following SCNT has been attributed to the upregulation of inflammatory cytokines resulting from abnormal expression of major histocompatibility complex class 1 proteins on the trophoblast of these conceptuses (Rutigliano *et al.* 2022). Rejection is likely to be considerably more problematic with a xenogeneic (from a different species) foetus. Furthermore, this may be a particular problem for those species with a haemochorial placental structure, including primates and some rodents, where maternal blood comes into direct contact with the foetal chorion. It has been proposed that separating the inner cell mass (ICM) from an endangered species' early embryo and injecting this into a trophoblast vesicle derived from the putative surrogate dam may overcome such problems of incompatibility (Saragusty *et al.* 2020). However, interactions between the trophoblast and ICM may pose challenges if these are derived from different species (Girgin *et al.* 2021). Furthermore, other potential issues of developing viable offspring cross-species include the role of exosomes (endosomal-derived membrane nanovesicles involved in intercellular communication (Zhang *et al.* 2019)) in implantation and early embryo development (Shi *et al.* 2021) and the acquisition of a suitable microbiome

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Table 5 Examples of mammalian species from which induced pluripotent stem (iPS) cells have been generated and their International Union for the Conservation of Nature (IUCN) red list status.

Species	IUCN red list status	Reference
Snow leopard (*Panthera uncia*)	Vulnerable	Verma *et al.* (2012)
Tiger (*Panthera tigris*)	Endangered	Verma *et al.* (2013)
Jaguar (*Panthera onca*)	Near threatened	Verma *et al.* (2013)
Serval (*Leptailurus serval*)	Least concern	Verma *et al.* (2013)
Somali wild ass (*Equus africanus somaliensis*)	Critically endangered	Ben-Nun *et al.* (2015)
Northern white rhinoceros (*Ceratotherium simum cottoni*)	Critically endangered	Ben-Nun *et al.* (2011)
Banteng (*Bos javanicus javanicus*)	Endangered	Ben-Nun *et al.* (2015)
Sumatran orangutan (*Pongo abelii*)	Critically endangered	Ramaswamy *et al.* (2015)
Drill (*Mandrillus leucophaeus*)	Endangered	Ben-Nun *et al.* (2011)
Chimpanzee (*Pan troglodytes*)	Endangered	Marchetto *et al.* (2013)
Bonobo (*Pan paniscus*)	Endangered	Marchetto *et al.* (2013)
Western gorilla (*Gorilla gorilla gorilla*)	Critically endangered	Wunderlich *et al.* (2014)
Prairie vole (*Microtus ochrogaster*)	Least concern	Manoli *et al.* (2012)
Naked mole-rat (*Heterocephalus glaber*)	Least concern	Lee *et al.* (2017)
Tasmanian devil (*Sarcophilus harrisii*)	Endangered	Weeratunga *et al.* (2018)
Little brown bat (*Myotis lucifugus*)	Endangered	Mo *et al.* (2014)
Platypus (*Ornithorhynchus anatinus*)	Near threatened	Whitworth *et al.* (2019)
Quail (*Coturnix*)	Least concern	Lu *et al.* (2015)
Zebra fish (*Danio rerio*)	Least concern	Peng *et al.* (2019)

for the development of immune function of the neonate (Macpherson *et al.* 2017). These and many other problems will need to be overcome in the future, and the fundamental importance of long-term cryobanking of live cells from endangered species needs to be underlined.

Conclusion

ART and aART have a huge potential for wildlife conservation, and therefore, many advances have been made in the last few decades. However, for these techniques to be applicable and therefore useful, prompt global cooperation and action are imperative. Knowledge sharing, data and sample inventory sharing and creating international networks of biobanks are paramount. Indeed, it is vital that protocols for all techniques are standardised, and for this, global collaboration is required. This is particularly important when research groups/biobanks only have access to very limited biological material to develop protocols on an *ad hoc* basis. Furthermore, viable tissue cryobanking should be considered in all future conservation strategies as a source of genetically diverse material that may be required in the future to combat the extinction crisis (Comizzoli & Holt 2019). Great progress is already being made to save endangered species by using ART and aART, as demonstrated by the successful captive propagation and reintroduction using ART of endangered Mississippi gopher frogs in the United States (Watt *et al.* 2021), efforts to breed cheetahs and other endangered felids via ART and aART (Wildt & Roth 1997) and the northern white rhino project to name just a few. However, despite the above example, the current focus is still primarily on mammalian taxa, and there is an urgent need for allowing these technologies to catch up across all others. These technologies open the way for more innovative conservation strategies and integration of traditional conservation methods with biologically based safety nets for species in danger. Focus needs to be placed on less charismatic taxa and the development of cryopreservation and storage protocols for tissues from avian, amphibian, reptilian, piscine and even invertebrate species in addition to the development of ART and aART for these taxa. This field of science is fast moving and vital to future biodiversity conservation efforts and will be a 'hot topic' as the ethical debate surrounding these technologies comes to the fore with the advent of new techniques and possibilities. We need to start banking samples before we lose more species, populations and genetic diversity; we do not know what will be needed in the future.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/RAF-22-0005.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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R L B drafted main body of text with additions from A M, M T P, A E B, J G and L M. R A provided Fig. 3. G J D, S L W and C H commented and edited text. All authors commented on and edited final version then approved for publication.

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References

Abe Y, Asano T, Ali M & Suzuki H 2010 Vitrification of canine cumulus-oocyte complexes in DAP213 with a cryotop holder. *Reproductive Medicine and Biology* **9** 115–120. (https://doi.org/10.1007/s12522-010-0045-6)

Allen CD, Burridge M, Mulhall S, Chafer ML, Nicolson VN, Pyne M, Zee YP, Jago SC, Lundie-Jenkins G, Holt WV, *et al.* 2008 Successful artificial insemination in the koala (*Phascolarctos cinereus*) using extended and extended-chilled semen collected by electroejaculation. *Biology of Reproduction* **78** 661–666. (https://doi.org/10.1095/biolreprod.107.064824)

Andrabi SMH & Maxwell WMC 2007 A review on reproductive biotechnologies for conservation of endangered mammalian species. *Animal Reproduction Science* **99** 223–243. (https://doi.org/10.1016/j.anireprosci.2006.07.002)

Andrae CS, Oliveira ECS, Ferraz MAMM & Nagashima JB 2021 Cryopreservation of grey wolf (*Canis lupus*) testicular tissue. *Cryobiology* **100** 173–179. (https://doi.org/10.1016/j.cryobiol.2021.01.010)

Appeltant R, Somfai T, Santos ECS, Dang-Nguyen TQ, Nagai T & Kikuchi K 2017 Effects of vitrification of cumulus-enclosed porcine oocytes at the germinal vesicle stage on cumulus expansion, nuclear progression and cytoplasmic maturation. *Reproduction, Fertility, and Development* **29** 2419–2429. (https://doi.org/10.1071/RD16386)

Arav A 2014 Cryopreservation of oocytes and embryos. *Theriogenology* **81** 96–102. (https://doi.org/10.1016/j.theriogenology.2013.09.011)

Arregui L, Rathi R, Zeng W, Honaramooz A, Gomendio M, Roldan ER & Dobrinski I 2008 Xenografting of adult mammalian testis tissue. *Animal Reproduction Science* **106** 65–76. (https://doi.org/10.1016/j.anireprosci.2007.03.026)

Asa CS, Bauman JE, Coonan TJ & Gray MM 2007 Evidence for induced estrus or ovulation in a canid, the island fox (*Urocyon littoralis*). *Journal of Mammalogy* **88** 436–440. (https://doi.org/10.1644/05-MAMM-A-295R2.1)

Asano A & Tajima A 2017 Development and preservation of avian sperm. *Advances in Experimental Medicine and Biology* **1001** 59–73. (https://doi.org/10.1007/978-981-10-3975-1_4)

Asturiano JF, Cabrita E & Horváth Á 2017 Progress, challenges and perspectives on fish gamete cryopreservation: a mini-review. *General and Comparative Endocrinology* **245** 69–76. (https://doi.org/10.1016/j.ygcen.2016.06.019)

AZA 2015 *Annual Report on Conservation and Science (Online)*. Silver Spring, Maryland. (available at: arcs_2015.pdf (speakcdn.com)). Accessed on 2 December 2021.

Bailey JL, Bilodeau JF & Cormier N 2000 Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. *Journal of Andrology* **21** 1–7.

Bailey JL, Lessard C, Jacques J, Brèque C, Dobrinski I, Zeng W & Galantino-Homer HL 2008 Cryopreservation of boar semen and its future importance to the industry. *Theriogenology* **70** 1251–1259. (https://doi.org/10.1016/j.theriogenology.2008.06.014)

Bajerski F, Bürger A, Glasmacher B, Keller ERJ, Müller K, Mühldorfer K, Nagel M, Rüdel H, Müller T, Schenkel J, *et al.* 2020 Factors determining microbial colonization of liquid nitrogen storage tanks used for archiving biological samples. *Applied Microbiology and Biotechnology* **104** 131–144. (https://doi.org/10.1007/s00253-019-10242-1)

Ballou JD 1984 Strategies for maintaining genetic diversity in captive populations through reproductive technology. *Zoo Biology* **3** 311–323. (https://doi.org/10.1002/zoo.1430030404)

Ballou JD, Lees C, Faust LJ, Long S, Lynch C, Bingaman Lackey L & Foose TJ 2010 Demographic and genetic management of captive populations Aug 15;219. In Wild mammals in captivity: principles and techniques for zoo management 2nd Edition. Eds DG Kleiman, KV Thompson and CK Baer. The University of Chicago Press

Benesova B & Trefil P 2016 Possibilities for preserving genetic resources in birds. *World's Poultry Science Journal* **72** 629–642. (https://doi.org/10.1017/S0043933916000489)

Benirschke K 1984 The frozen zoo concept. *Zoo Biology* **3** 325–328. (https://doi.org/10.1002/zoo.1430030405)

Ben-Nun IF, Montague SC, Houck ML, Tran HT, Garitaonandia I, Leonardo TR, Wang YC, Charter SJ, Laurent LC, Ryder OA, *et al.* 2011 Induced pluripotent stem cells from highly endangered species. *Nature Methods* **8** 829–831. (https://doi.org/10.1038/nmeth.1706)

Ben-Nun IF, Montague SC, Houck ML, Ryder O & Loring JF 2015 Generation of induced pluripotent stem cells from mammalian endangered species. In *Cell Reprogramming: Methods and Protocols*. Eds **PJ Verma & H Sumer**. New York, NY: Springer.

Bhak JS, Lee SL, Ock SA, Mohana Kumar B, Choe SY & Rho GJ 2006 Developmental rate and ploidy of embryos produced by nuclear transfer with different activation treatments in cattle. *Animal Reproduction Science* **92** 37–49. (https://doi.org/10.1016/j.anireprosci.2005.04.016)

Bhat MH, Sharma V, Khan FA, Naykoo NA, Yaqoob SH, Ruby K, Khan HM, Fazili MR, Ganai NA & Shah RA 2014 Comparison of slow freezing and vitrification on ovine immature oocytes. *Cryo Letters* **35** 77–82.

Bishop PJ, Angulo A, Lewis JP, Moore RD, Rabb GB & Moreno JG 2012 The Amphibian Extinction Crisis – what will it take to put the action into the Amphibian Conservation Action Plan? *Sapiens* **5** 97–111.

Blesbois E, Grasseau I & Seigneurin F 2005 Membrane fluidity and the ability of domestic bird spermatozoa to survive cryopreservation. *Reproduction* **129** 371–378. (https://doi.org/10.1530/rep.1.00454)

Borges AA & Pereira AF 2019 Potential role of intraspecific and interspecific cloning in the conservation of wild mammals. *Zygote* **27** 111–117. (https://doi.org/10.1017/S0967199419000170)

Borges AA, Santos MVO, Nascimento LE, Lira GPO, Praxedes ÉA, Oliveira MF, Silva AR & Pereira AF 2020 Production of collared peccary (Pecari tajacu Linnaeus, 1758) parthenogenic embryos following different oocyte chemical activation and in vitro maturation conditions. *Theriogenology* **142** 320–327.

Borini A & Bianchi V 2012 Oocyte cryopreservation. In *Fertility Preservation*. Eds E Seli and A Agarwal

Boutelle S, Lenahan K, Krisher R, Bauman KL, Asa CS & Silber S 2011 Vitrification of oocytes from endangered Mexican gray wolves (*Canis lupus baileyi*). *Theriogenology* **75** 647–654. (https://doi.org/10.1016/j.theriogenology.2010.10.004)

Briski O & Salamone DF 2022 Past, present and future of ICSI in livestock species. *Animal Reproduction Science* In press. (https://doi.org/10.1016/j.anireprosci.2022.106925)

Brown J L, Goritz F, Pratt-Hawkes N, Hermes R, Galloway M, Graham L H, Gray C, Walker S L, Gomez A, Moreland R, et al. 2004. Successful artificial insemination of an Asian elephant at the National Zoological Park. *Zoo Biology* **23** 45-63.

Browne RK, Clulow J & Mahony M 2001 Short-term storage of cane toad (*Bufo marinus*) gametes. *Reproduction* **121** 167–173. (https://doi.org/10.1530/rep.0.1210167)

Browne RK, Clulow J & Mahony M 2002 The short-term storage and cryopreservation of spermatozoa from hylid and myobatrachid frogs. *Cryo Letters* **23** 129–136.

Browne RK, Seratt J, Vance C& Kouba A 2006 Hormonal priming, induction of ovulation and in-vitro fertilization of the endangered Wyoming toad (*Bufo baxteri*). *Reproductive Biology and Endocrinology* **4** 34. (https://doi.org/10.1186/1477-7827-4-34)

Browne RK, Silla AJ, Upton R, Della-Togna G, Marcec-Greaves R, Shishova NV, Uteshev VK, Proaño B, Pérez OD, Mansour N, *et al.* 2019 Sperm collection and storage for the sustainable management of amphibian biodiversity. *Theriogenology* **133** 187–200. (https://doi.org/10.1016/j.theriogenology.2019.03.035)

Byers O, Lees C, Wilcken J & Schwitzer C 2013 The one plan approach: the philosophy and implementation of CBSG's approach to integrated species conservation planning. *WAZA Magazine* **14** 2–5. (available at: www.waza.org/en/site/conservation/integrated-species-conservation)

Byrne PG & Silla AJ 2010 Hormonal induction of gamete release, and *in-vitro* fertilisation, in the critically endangered southern corroboree frog, *Pseudophryne corroboree*. *Reproductive Biology and Endocrinology* **8** 144. (https://doi.org/10.1186/1477-7827-8-144)

Carballo L, Battistotti A, Teltscher K, Lierz M, Bublat A, Valcu M & Kempenaers B 2019 Sperm morphology and evidence for sperm competition among parrots. *Journal of Evolutionary Biology* **32** 856–867. (https://doi.org/10.1111/jeb.13487)

Cardoso B, Sánchez-Ajofrín I, Castaño C, García-Álvarez O, Esteso MC, Maroto-Morales A, Iniesta-Cuerda M, Garde JJ, Santiago-Moreno J & Soler AJ 2020 Optimization of sperm cryopreservation protocol for peregrine falcon (*Falco peregrinus*). *Animals* **10** 691. (https://doi.org/10.3390/ani10040691)

Ceballos G & Ehrlich PR 2018 The misunderstood sixth mass extinction. *Science* **360** 1080–1081.

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM & Palmer TM 2015 Accelerated modern human-induced species losses: entering the sixth mass extinction. *Science Advances* **1** e1400253. (https://doi.org/10.1126/sciadv.1400253)

Chatdarong K 2011 Gamete rescues from gonads of wild animal post mortem. *Thai Journal of Veterinary Medicine* **41** 99–102.

Che-Castaldo J, Gray SM, Rodriguez-Clark KM, Schad Eebes K & Faust LJ 2021 Expected demographic and genetic declines not found in most zoo and aquarium populations. *Frontiers in Ecology and the Environment* **19** 435–442. (https://doi.org/10.1002/fee.2362)

Chemnick LG, Houck ML & Ryder OA 2009 Banking of genetic resources: the Frozen Zoo® at the San Diego Zoo. In *Conservation Genetics in the Age of Genomics*, pp. 124–130. Eds **G Amato, R Desalle, HC Rosenblum & OA Ryder**. New York, NY, USA: Columbio University Press.

Cheng TL, Rovito SM, Wake DB & Vredenburg VT 2011 Coincident mass extirpation of Neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *PNAS* **108** 9502–9507. (https://doi.org/10.1073/pnas.1105538108)

Chian RC, Kuwayama M, Tan L, Tan J, Kato O & Nagai T 2004 High survival rate of bovine oocytes matured in vitro following vitrification. *Journal of Reproduction and Development* **50** 685–696. (https://doi.org/10.1262/jrd.50.685)

Choudhary KK, Kavya KM, Jerome A & Sharma RK 2016 Advances in reproductive biotechnologies. *Veterinary World* **9** 388–395. (https://doi.org/10.14202/vetworld.2016.388-395)

Clarke AG 2009 The Frozen Ark Project: the role of zoos and aquariums in preserving the genetic material of threatened animals. *International Zoo Yearbook* **43** 222–230. (https://doi.org/10.1111/j.1748-1090.2008.00074.x)

Clérico G, Taminelli G, Veronesi JC, Polola J, Pagura N, Pinto C & Sansinena M 2021 Mitochondrial function, blastocyst development and live foals born after ICSI of immature vitrified/warmed equine oocytes matured with or without melatonin. *Theriogenology* **160** 40–49. (https://doi.org/10.1016/j.theriogenology.2020.10.036)

Clulow J & Clulow S 2016 Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed. *Reproduction, Fertility, and Development* **28** 1116–1132. (https://doi.org/10.1071/RD15466)

Clulow J, Upton R, Trudeau VL & Clulow S 2019*a* Amphibian assisted reproductive technologies: moving from technology to application. In *Reproductive Sciences in Animal Conservation. Advances in Experimental Medicine and Biology*, vol. 1200, pp. 413–463. Eds **P Comizzoli, J Brown & W Holt**. Cham: Springer. (https://doi.org/10.1007/978-3-030-23633-5_14)

Clulow J, Upton R, Trudeau VL & Clulow S 2019*b* Amphibian assisted reproductive technologies: moving from technology to application. *Advances in Experimental Medicine and Biology* **1200** 413–463. (https://doi.org/10.1007/978-3-030-23633-5_14)

Comizzoli P 2015 Biobanking efforts and new advances in male fertility preservation for rare and endangered species. *Asian Journal of Andrology* **17** 640–645. (https://doi.org/10.4103/1008-682X.153849)

Comizzoli P 2020 Birth of a giant panda cub after artificial insemination with frozen-thawed semen: a powerful reminder about the key role of biopreservation and biobanking for wildlife conservation. *Biopreservation and Biobanking* **18** 349–350. (https://doi.org/10.1089/bio.2020.29076.pjc)

Comizzoli P & Holt WV 2019 Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. *Biology of Reproduction* **101** 514–525. (https://doi.org/10.1093/biolre/ioz031)

Comizzoli P & Wildt DE 2017 Cryobanking biomaterials from wild animal species to conserve genes and biodiversity: relevance to human biobanking and biomedical research. In *Biobanking of Human Biospecimens: Principles and Practice*. Eds **P Hainaut, J Vaught, K Zatloukal & M Pasterk**. Cham: Springer International Publishing.

Comizzoli P, Songsasen N, Hagedorn M & Wildt DE 2012 Comparative cryobiological traits and requirements for gametes and gonadal tissues collected from wildlife species. *Theriogenology* **78** 1666–1681. (https://doi.org/10.1016/j.theriogenology.2012.04.008)

Comizzoli P, Brown JL & Holt WV 2019 Reproductive science as an essential component of conservation biology: new edition. In *Reproductive Sciences in Animal Conservation*. Eds **P Comizzoli, JL Brown & WV Holt**. Cham: Springer International Publishing.

Conforti VA, Adania CH, Gonzalez PG, De Oliveira C & Swanson WF 2009 155 Novel recipient synchronization regimens for successful embryo transfer in the Brazilian ocelot following long-term frozen embryo storage. *Reproduction, Fertility and Development* **21** 176–177. (https://doi.org/10.1071/RDv21n1Ab155)

CPSG 2016 Prioritizing the collection of samples for genetic rescue. In *IUCN CPSG Annual Meeting*. Gland, Switzerland: IUCN SSC Conservation Planning Specialist Group.

Crabbé E, Verheyen G, Tournaye H & Van Steirteghem A 1997 The use of enzymatic procedures to recover testicular germ cells. *Human Reproduction* **12** 1682–1687. (https://doi.org/10.1093/humrep/12.8.1682)

Curry MR 2000 Cryopreservation of semen from domestic livestock. *Reviews of Reproduction* **5** 46–52. (https://doi.org/10.1530/ror.0.0050046)

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-22-0005

Da Silva AM, Bezerra LGP, Praxedes ECG, Moreira SSJ, De Souza CMP, De Oliveira MF, Pereira AF, Comizzoli P & Silva AR 2019 Combination of intracellular cryoprotectants preserves the structure and the cells proliferative capacity potential of adult collared peccary testicular tissue subjected to solid surface vitrification. *Cryobiology* **91** 53–60. (https://doi.org/10.1016/j.cryobiol.2019.10.199)

Damiani P, Gomez M, Cole A, Pope E, Aguilar R, Hammond B, Nel L, Cortez C, Vaccaro J, Sarrat E, *et al.* 2004 The production of intracytoplasmic sperm injection lion (*Panthera leo*) embyros using spermatozoa collected by percutaneous epididymal sperm aspiration from vasectomized males. *Reproduction, Fertility and Development* **16** 223–224. (https://doi.org/10.1071/RDv16n1Ab204)

De Mori B, Spiriti MM, Pollastri I, Normando S, Biasetti P, Florio D, Andreucci F, Colleoni S, Galli C, Göritz F, *et al.* 2021 An ethical assessment tool (ETHAS) to evaluate the application of assisted reproductive technologies in mammals' conservation: the case of the northern white rhinoceros (*Ceratotherium simum cottoni*). *Animals* **11** 312. (https://doi.org/10.3390/ani11020312)

Diwan AD, Harke SN, Panche AN & Panche AN 2020 Cryobanking of fish and shellfish egg, embryos and larvae: an overview. *Frontiers in Marine Science* **7** 251. (https://doi.org/10.3389/fmars.2020.00251)

Doudna JA & Charpentier E 2014 Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science* **346** 1258096. (https://doi.org/10.1126/science.1258096)

Eppig JJ & O'Brien MJ 1996 Development *in vitro* of mouse oocytes from primordial follicles. *Biology of Reproduction* **54** 197–207. (https://doi.org/10.1095/biolreprod54.1.197)

Fayomi AP, Peters K, Sukhwani M, Valli-Pulaski H, Shetty G, Meistrich ML, Houser L, Robertson N, Roberts V, Ramsey C, *et al.* 2019 Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. *Science* **363** 1314–1319. (https://doi.org/10.1126/science.aav2914)

Fernandez-Gonzalez L & Jewgenow K 2017 Cryopreservation of feline oocytes by vitrification using commercial kits and slush nitrogen technique. *Reproduction in Domestic Animals* **52** (Supplement 2) 230–234. (https://doi.org/10.1111/rda.12837)

Fernandez-Gonzalez L, Huebinger J & Jewgenow K 2021 Comparison of different materials for self-pressurized vitrification of feline oocytes – first results. *Animals* **11** 1314. (https://doi.org/10.3390/ani11051314)

Ficetola GF, Rondinini C, Bonardi A, Baisero D & Padoa-Schioppa E 2015 Habitat availability for amphibians and extinction threat: a global analysis. *Diversity and Distributions* **21** 302–311. (https://doi.org/10.1111/ddi.12296)

Figueiredo JR, Hulshof SCJ, Van Den Hurk R, Ectors FJ, Fontes RS, Nusgens B, Bevers MM & Beckers JF 1993 Development of a combined new mechanical and enzymatic method for the isolation of intact preantral follicles from fetal, calf and adult bovine ovaries. *Theriogenology* **40** 789–799. (https://doi.org/10.1016/0093-691x(93)90214-p)

Figueiredo JR, Hulshof SCJ, Van Den Hurk R, Bevers MM, Thiry M, Nusgens B & Beckers JF 1994*a* The physiological status of the ovarian donor affects in vitro development of isolated bovine preantral follicles. *Theriogenology* **42** 1303–1310. (https://doi.org/10.1016/0093-691X(94)90250-M)

Figueiredo JR, Hulshof SCJ, Van Den Hurk R, Nusgens B, Bevers MM, Ectors FJ & Beckers JF 1994*b* Preservation of oocyte and granulosa cell morphology in bovine preantral follicles cultured in vitro. *Theriogenology* **41** 1333–1346. (https://doi.org/10.1016/0093-691x(94)90492-2)

Foden WB, Butchart SH, Stuart SN, Vié JC, Akçakaya HR, Angulo A, Devantier LM, Gutsche A, Turak E, Cao L, *et al.* 2013 Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. *PLoS ONE* **8** e65427. (https://doi.org/10.1371/journal.pone.0065427)

Folch J, Cocero MJ, Chesné P, Alabart JL, Domínguez V, Cognié Y, Roche A, Fernández-Árias A, Martí JI, Sánchez P, *et al.* 2009 First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. *Theriogenology* **71** 1026–1034. (https://doi.org/10.1016/j.theriogenology.2008.11.005)

Friedrich Ben-Nun IF, Montague SC, Houck ML, Tran HT, Garitaonandia I, Leonardo TR, Wang YC, Charter SJ, Laurent LC, Ryder OA, *et al.* 2011 Induced pluripotent stem cells from highly endangered species. *Nature Methods* **8** 829–831. (https://doi.org/10.1038/nmeth.1706)

Fuku E, Kojima T, Shioya Y, Marcus GJ & Downey BR 1992 In vitro fertilization and development of frozen-thawed bovine oocytes. *Cryobiology* **29** 485–492. (https://doi.org/10.1016/0011-2240(92)90051-3)

Fuller BJ 2004 Cryoprotectants: the essential antifreezes to protect life in the frozen state. *Cryo Letters* **25** 375–388.

Fuller B & Paynter S 2004 Fundamentals of cryobiology in reproductive medicine. *Reproductive Biomedicine Online* **9** 680–691. (https://doi.org/10.1016/s1472-6483(10)61780-4)

García-Martínez T, Mogas T, Mullen SF, Martínez-Rodero I, Gulieva RE & Higgins AZ 2021 Effect of cryoprotectant concentration on bovine oocyte permeability and comparison of two membrane permeability modelling approaches. *Scientific Reports* **11** 15387. (https://doi.org/10.1038/s41598-021-94884-0)

Gastal GDA, Aguiar FLN, Rodrigues APR, Scimeca JM, Apgar GA, Banz WJ, Feugang JM & Gastal EL 2018 Cryopreservation and in vitro culture of white-tailed deer ovarian tissue. *Theriogenology* **113** 253–260. (https://doi.org/10.1016/j.theriogenology.2018.03.003)

Gillis AB, Guy EL, Kouba AJ, Allen PJ, Marcec-Greaves RM & Kouba CK 2021*a* Short-term storage of tiger salamander (*Ambystoma tigrinum*) spermatozoa: the effect of collection type, temperature and time. *PLoS ONE* **16** e0245047.

Gillis JD, Penfold LM & Mylniczenko ND 2021*b* Initial characterization of male southern stingray (*Hypanus americanus*) reproductive parameters and preliminary investigation of sperm cryopreservation. *Animals* **11** 2716. (https://doi.org/10.3390/ani11092716)

Girgin MU, Broguiere N, Hoehnel S, Brandenberg N, Mercier B, Arias AM & Lutolf MP 2021 Bioengineered embryoids mimic post-implantation development *in vitro*. *Nature Communications* **12** 5140. (https://doi.org/10.1038/s41467-021-25237-8)

Gómez MC, Pope CE, Giraldo A, Lyons LA, Harris RF, King AL, Cole A, Godke RA & Dresser BL 2004 Birth of African wildcat cloned kittens born from domestic cats. *Cloning and Stem Cells* **6** 247–258. (https://doi.org/10.1089/clo.2004.6.247)

Gómez MC, Pope CE, Kutner RH, Ricks DM, Lyons LA, Ruhe M, Dumas C, Lyons J, López M, Dresser BL, *et al.* 2008 Nuclear transfer of sand cat cells into enucleated domestic cat oocytes is affected by cryopreservation of donor cells. *Cloning and Stem Cells* **10** 469–483. (https://doi.org/10.1089/clo.2008.0021)

Gouveia C, Huyser C, Egli D & Pepper MS 2020 Lessons learned from somatic cell nuclear transfer. *International Journal of Molecular Sciences* **21** 2314. (https://doi.org/10.3390/ijms21072314)

Guan WJ, Liu CQ, Li CY, Liu D, Zhang WX & Ma YH 2010 Establishment and cryopreservation of a fibroblast cell line derived from Bengal tiger (*Panthera tigris tigris*). *Cryo Letters* **31** 130–138.

Guenther JF, Seki S, Kleinhans FW, Edashige K, Roberts DM & Mazur P 2006 Extra- and intra-cellular ice formation in stage I and II *Xenopus laevis* oocytes. *Cryobiology* **52** 401–416. (https://doi.org/10.1016/j.cryobiol.2006.02.002)

Gurdon JB, Laskey RA & Reeves OR 1975 The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *Journal of Embryology and Experimental Morphology* **34** 93–112. (https://doi.org/10.1242/dev.34.1.93)

Hagedorn M, Carter V, Martorana K, Paresa MK, Acker J, Baums IB, Borneman E, Brittsan M, Byers M, Henley M, *et al.* 2012 Preserving and using germplasm and dissociated embryonic cells for conserving Caribbean and Pacific coral. *PLoS ONE* **7** e33354. (https://doi.org/10.1371/journal.pone.0033354)

Hagedorn M, Carter VL, Henley EM, Van Oppen MJH, Hobbs R & Spindler RE 2017 Producing coral offspring with cryopreserved sperm: a tool for coral reef restoration. *Scientific Reports* **7** 14432. (https://doi.org/10.1038/s41598-017-14644-x)

Hagedorn M, Page CA, Oneill K, Flores DM, Tichy L, Chamberland VF, Lager C, Zuchowicz N, Lohr K, Blackburn H, *et al.* 2018 Successful demonstration of assisted gene flow in the threatened coral Acropora palmata across genetically-isolated Caribbean populations using cryopreserved sperm. *bioRxiv* 492447. (https://doi.org/10.1101/492447)

Hagedorn M, Spindler R & Daly J 2019 Cryopreservation as a tool for reef restoration: 2019. *Advances in Experimental Medicine and Biology* **1200** 489–505. (https://doi.org/10.1007/978-3-030-23633-5_16)

Hajian M, Hosseini SM, Forouzanfar M, Abedi P, Ostadhosseini S, Hosseini L, Moulavi F, Gourabi H, Shahverdi AH, Vosough Taghi Dizaj A, *et al.* 2011 'Conservation cloning' of vulnerable Esfahan mouflon (*Ovis orientalis isphahanica*): *in vitro* and *in vivo* studies. *European Journal of Wildlife Research* **57** 959–969. (https://doi.org/10.1007/s10344-011-0510-5)

Hamano S, Koikeda A, Kuwayama M & Nagai T 1992 Full-term development of in vitro-matured, vitrified and fertilized bovine oocytes. *Theriogenology* **38** 1085–1090. (https://doi.org/10.1016/0093-691x(92)90122-8)

Hamazaki N, Kyogoku H, Araki H, Miura F, Horikawa C, Hamada N, Shimamoto S, Hikabe O, Nakashima K, Kitajima TS, *et al.* 2021 Reconstitution of the oocyte transcriptional network with transcription factors. *Nature* **589** 264–269. (https://doi.org/10.1038/s41586-020-3027-9)

Herrick JR 2019 Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. *Biology of Reproduction* **100** 1158–1170. (https://doi.org/10.1093/biolre/ioz025)

Hewitt R & Watson P 2013 Defining biobank. *Biopreservation and Biobanking* **11** 309–315. (https://doi.org/10.1089/bio.2013.0042)

Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, Shimamoto S, Imamura T, Nakashima K, Saitou M, *et al.* 2016 Reconstitution *in vitro* of the entire cycle of the mouse female germ line. *Nature* **539** 299–303. (https://doi.org/10.1038/nature20104)

Hildebrandt TB, Hermes R, Goeritz F, Appeltant R, Colleoni S, De Mori B, Diecke S, Drukker M, Galli C, Hayashi K, *et al.* 2021 The ART of bringing extinction to a freeze–History and future of species conservation, exemplified by rhinos. *Theriogenology* **169** 76–88. (https://doi.org/10.1016/j.theriogenology.2021.04.006)

Hinkle K, Orwig KE, Valli-Pulaski H, Taylor S, Van Leeuwen K, Carpentieri D & Walsh A 2021 Cryopreservation of ovarian tissue for pediatric fertility. *Biopreservation and Biobanking* **19** 130–135. (https://doi.org/10.1089/bio.2020.0124)

Hoban S, Bruford M, D'Urban Jackson J, Lopes-Fernandes M, Heuertz M, Hohenlohe PA, Paz-Vinas I, Sjögren-Gulve P, Segelbacher G, Vernesi C, *et al.* 2020 Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation* **248** 108654. (https://doi.org/10.1016/j.biocon.2020.108654)

Hobbs RJ, O'Brien JK & Spindler RE 2019 Strategic gene banking for conservation: the ins and outs of a living bank. In *Scientific Foundations of Zoos and Aquariums: Their Role in Conservation and Research*. Eds **AB Kaufman, MJ Bashaw & TL Maple**. Cambridge: Cambridge University Press.

Holt WV 2000 Fundamental aspects of sperm cryobiology: the importance of species and individual differences. *Theriogenology* **53** 47–58. (https://doi.org/10.1016/s0093-691x(99)00239-3)

Holt WV & Lloyd RE 2009 Artificial insemination for the propagation of CANDES: the reality! *Theriogenology* **71** 228–235. (https://doi.org/10.1016/j.theriogenology.2008.09.003)

Holt WV, Pickard AR & Prather RS 2004 Wildlife conservation and reproductive cloning. *Reproduction* **127** 317–324. (https://doi.org/10.1530/rep.1.00074)

Houck ML 2019 Fibroblast cell culture and cryopreservation of endangered vertebrates. *Cryobiology* **91** 153–154. (https://doi.org/10.1016/j.cryobiol.2019.10.037)

Howard JG, Lynch C, Santymire RM, Marinari PE & Wildt DE 2016 Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. *Animal Conservation* **19** 102–111. (https://doi.org/10.1111/acv.12229)

Howell LG, Frankham R, Rodger JC, Witt RR, Clulow S, Upton RMO & Clulow J 2021 Integrating biobanking minimises inbreeding and produces significant cost benefits for a threatened frog captive breeding programme. *Conservation Letters* **14** e12776. (https://doi.org/10.1111/conl.12776)

Hübner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R, Wood J, Strauss JF, 3rd, Boiani M & Schöler HR 2003 Derivation of oocytes from mouse embryonic stem cells. *Science* **300** 1251–1256. (https://doi.org/10.1126/science.1083452)

Huffmeyer AA, Sikich JA, Vickers TW, Riley SP & Wayne RK 2022 First reproductive signs of inbreeding depression in Southern California male mountain lions (Puma concolor). *Theriogenology* **177** 157–164. (https://doi.org/10.1016/j.theriogenology.2021.10.016)

Hulshof SCJ, Figueiredo JR, Beckers JF, Bevers MM, Van Der Donk JA & Van Den Hurk R 1995 Effects of fetal bovine serum, FSH and 17β-estradiol on the culture of bovine preantral follicles. *Theriogenology* **44** 217–226. (https://doi.org/10.1016/0093-691x(95)00171-4)

Hunt CJ 2017 Cryopreservation: vitrification and controlled rate cooling. *Methods in Molecular Biology* **1590** 41–77. (https://doi.org/10.1007/978-1-4939-6921-0_5)

Hurtt AE, Landim-Alvarenga F, Seidel Jr GE & Squires EL 2000 Vitrification of immature and mature equine and bovine oocytes in an ethylene glycol, Ficoll and sucrose solution using open-pulled straws. *Theriogenology* **54** 119–128. (https://doi.org/10.1016/s0093-691x(00)00330-7)

Hwang I, Jeong YW, Kim JJ, Lee HJ, Kang M, Park KB, Park JH, Kim YW, Kim WT, Shin T, *et al.* 2012 Successful cloning of coyotes through interspecies somatic cell nuclear transfer (iSCNT) using domestic dog oocytes. *Biology of Reproduction* **87** 56–56.

Imbler S 2021 *Meet Elizabeth Ann, The First Cloned Black-Footed Ferret (Online)*. The New York Times. (available at: https://www.nytimes.com/2021/02/18/science/black-footed-ferret-clone.html). Accessed on 16 November 2021.

Inoue K, Wakao H, Ogonuki N, Miki H, Seino K, Nambu-Wakao R, Noda S, Miyoshi H, Koseki H, Taniguchi M, *et al.* 2005 Generation of cloned mice by direct nuclear transfer from natural killer T cells. *Current Biology* **15** 1114–1118. (https://doi.org/10.1016/j.cub.2005.05.021)

Ishikura Y, Ohta H, Sato T, Murase Y, Yabuta Y, Kojima Y, Yamashiro C, Nakamura T, Yamamoto T, Ogawa T, *et al.* 2021 *In vitro* reconstitution of the whole male germ-cell development from mouse pluripotent stem cells. *Cell Stem Cell* **28** 2167.e9–2179.e9. (https://doi.org/10.1016/j.stem.2021.08.005)

IUCN 2016 *IUCN SSC Guiding Principles on Creating Proxies of Extinct Species*. IUCN.

IUCN 2021 *IUCN Red List of Threatened Species (Online)*. IUCN. Accessed 2021.

IUCN SSC Amphibian Specialist Group 2017 Leptodactylus fallax. *The IUCN Red List of Threatened Species* **2017** e.T57125A3055585. (https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T57125A3055585.en). Accessed on 21 January 2022.

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-22-0005

Janssen DL, Edwards ML, Koster JA, Lanza RP & Ryder OA 2004 Postnatal management of chryptorchid banteng calves cloned by nuclear transfer utilizing frozen fibroblast cultures and enucleated cow ova. *Reproduction, Fertility and Development* **16** 224–224. (https://doi.org/10.1071/RDv16n1Ab206)

Jeon Y, Nam YH, Cheong SA, Kwak SS, Lee E & Hyun SH 2016 Absence of nucleolus formation in raccoon dog-porcine interspecies somatic cell nuclear transfer embryos results in embryonic developmental failure. *Journal of Reproduction and Development* **62** 345–350. (https://doi.org/10.1262/jrd.2015-175)

Jewgenow K & Zahmel J 2020 Preservation of female genetic resources in feline species. *Theriogenology* **156** 124–129. (https://doi.org/10.1016/j.theriogenology.2020.06.040)

Jia BY, Xiang DC, Quan GB, Zhang B, Shao QY, Hong QH & Wu GQ 2019 Transcriptome analysis of porcine immature oocytes and surrounding cumulus cells after vitrification and in vitro maturation. *Theriogenology* **134** 90–97. (https://doi.org/10.1016/j.theriogenology.2019.05.019)

Jia B, Xiang D, Fu X, Shao Q, Hong Q, Quan G & Wu G 2020 Proteomic changes of porcine oocytes after vitrification and subsequent *in vitro* maturation: a tandem mass tag-based quantitative analysis. *Frontiers in Cell and Developmental Biology* **8** 614577. (https://doi.org/10.3389/fcell.2020.614577)

Joaquim DC, Borges ED, Viana IGR, Navarro PA & Vireque AA 2017 Risk of contamination of gametes and embryos during cryopreservation and measures to prevent cross-contamination. *BioMed Research International* **2017** 1840417. (https://doi.org/10.1155/2017/1840417)

Johnston SD & Holt WV 2019 Using the koala (*Phascolarctos cinereus*) as a case study to illustrate the development of artificial breeding technology in marsupials: an update. In *Reproductive Sciences in Animal Conservation*. Eds **P Comizzoli, JL Brown & WV Holt**. Cham: Springer International Publishing.

Johnson JA, Altwegg R, Evans DM, Ewen JG, Gordon IJ, Pettorelli N & Young JK 2016 Is there a future for genome-editing technologies in conservation? *Animal Conservation* **19** 97–101. (https://doi.org/10.1111/acv.12273)

Kaneko T, Ito H, Sakamoto H, Onuma M & Inoue-Murayama M. 2014 Sperm preservation by freeze-drying for the conservation of wild animals. *PloS one* **9** e113381. (https://doi.org/10.1371/journal.pone.0113381)

Keogh LM, Byrne PG & Silla AJ 2017 The effect of gentamicin on sperm motility and bacterial abundance during chilled sperm storage in the Booroolong frog. *General and Comparative Endocrinology* **243** 51–59. (https://doi.org/10.1016/j.ygcen.2016.11.005)

Kochan J, Nižański W, Moreira N, Cubas ZS, Nowak A, Prochowska S, Partyka A, Młodawska W & Skotnicki J 2019 ARTs in wild felid conservation programmes in Poland and in the world. *Journal of Veterinary Research* **63** 457–464. (https://doi.org/10.2478/jvetres-2019-0043)

Kong HS, Hong YH, Lee J, Youm HW, Lee JR, Suh CS & Kim SH 2021 Antifreeze protein supplementation during the warming of vitrified bovine ovarian tissue can improve the ovarian tissue quality after xenotransplantation. *Frontiers in Endocrinology* **12** 672619. (https://doi.org/10.3389/fendo.2021.672619)

Korody ML, Ford SM, Nguyen TD, Pivaroff CG, Valiente-Alandi I, Peterson SE, Ryder OA & Loring JF 2021 Rewinding extinction in the northern white rhinoceros: genetically diverse induced pluripotent stem cell bank for genetic rescue. *Stem Cells and Development* **30** 177–189. (https://doi.org/10.1089/scd.2021.0001)

Kouba A, Willis E, Vance CK, Hasenstab S, Reichling S, Krebs J, Linhoff L, Snoza M, Langhorne C & Germano J 2012 116 Development of assisted reproduction technologies for the endangered Mississippi gopher frog (*rana sevosa*) and sperm transfer for *in vitro* fertilization. *Reproduction, Fertility and Development* **24** 170. (https://doi.org/10.1071/RDv24n1Ab116)

Kouba AJ, Lloyd RE, Houck ML, Silla AJ, Calatayud N, Trudeau VL, Clulow J, Molinia F, Langhorne C, Vance C, *et al.* 2013 Emerging trends for biobanking amphibian genetic resources: the hope, reality and challenges for the next decade. *Biological Conservation* **164** 10–21. (https://doi.org/10.1016/j.biocon.2013.03.010)

Lagutina I, Fulka H, Lazzari G & Galli C 2013 Interspecies somatic cell nuclear transfer: advancements and problems. *Cellular Reprogramming* **15** 374–384. (https://doi.org/10.1089/cell.2013.0036)

Lanza RP, Cibelli JB, Diaz F, Moraes CT, Farin PW, Farin CE, Hammer CJ, West MD & Damiani P 2000 Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning* **2** 79–90. (https://doi.org/10.1089/152045500436104)

Lanzendorf SE, Holmgren WJ, Schaffer N, Hatasaka H, Wentz AC & Jeyendran RS 1992 In vitro fertilization and gamete micromanipulation in the lowland gorilla. *Journal of Assisted Reproduction and Genetics* **9** 358–364. (https://doi.org/10.1007/BF01203960)

Lawson B, Clulow S, Mahony MJ & Clulow J 2013 Towards gene banking amphibian maternal germ lines: short-term incubation, cryoprotectant tolerance and cryopreservation of embryonic cells of the frog, *Limnodynastes peronei*. *PLoS ONE* **4** e60760.

Lee WY, Park HJ, Lee R, Lee KH, Kim YH, Ryu BY, Kim NH, Kim JH, Kim JH, Moon SH, *et al.* 2013 Establishment and *in vitro* culture of porcine spermatogonial germ cells in low temperature culture conditions. *Stem Cell Research* **11** 1234–1249. (https://doi.org/10.1016/j.scr.2013.08.008)

Lee SG, Mikhalchenko AE, Yim SH, Lobanov AV, Park JK, Choi KH, Bronson RT, Lee CK, Park TJ & Gladyshev VN 2017 Naked mole rat induced pluripotent stem cells and their contribution to interspecific chimera. *Stem Cell Reports* **9** 1706–1720. (https://doi.org/10.1016/j.stemcr.2017.09.013)

Lee J, Lee Y, Lee GS, Lee ST & Lee E 2019 Comparative study of the developmental competence of cloned pig embryos derived from spermatogonial stem cells and fetal fibroblasts. *Reproduction of Domestic Animals* **54** 1258–1264. (https://doi.org/10.1111/rda.13507)

Lee AR, Park JH, Shim SH, Hong K, La H, Park KS & Lee DR 2021 Genome stabilization by RAD51-stimulatory compound 1 enhances efficiency of somatic cell nuclear transfer-mediated reprogramming and full-term development of cloned mouse embryos. *Cell Proliferation* **54** e13059. (https://doi.org/10.1111/cpr.13059)

Lees CM & Wilcken J 2009 Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook* **43** 6–18. (https://doi.org/10.1111/j.1748-1090.2008.00066.x)

Lees C & Wilcken J 2011 Global programmes for sustainability. *WAZA Magazine* **12** 2–5.

Leibo SP 1980 Water permeability and its activation energy of fertilized and unfertilized mouse ova. *Journal of Membrane Biology* **53** 179–188. (https://doi.org/10.1007/BF01868823)

Leibo SP & Songsasen N 2002 Cryopreservation of gametes and embryos of non-domestic species. *Theriogenology* **57** 303–326. (https://doi.org/10.1016/s0093-691x(01)00673-2)

Li LF, Yue H, Ma J, Guan WJ & Ma YH 2009 Establishment and characterization of a fibroblast line from Simmental cattle. *Cryobiology* **59** 63–68. (https://doi.org/10.1016/j.cryobiol.2009.04.009)

Li P, Hu H, Yang S, Tian R, Zhang Z, Zhang W, Ma M, Zhu Y, Guo X, Huang Y, *et al.* 2013 Differentiation of induced pluripotent stem cells into male germ cells *in vitro* through embryoid body formation and retinoic acid or testosterone induction. *BioMed Research International* **2013** 608728. (https://doi.org/10.1155/2013/608728)

Lima GL, Luz VB, Lunardi FO, Souza ALP, Peixoto GCX, Rodrigues APR, Oliveira MF, Santos RR & Silva AR 2019 Effect of cryoprotectant type and concentration on the vitrification of collared peccary (*Pecari tajacu*) ovarian tissue. *Animal Reproduction Science* **205** 126–133. (https://doi.org/10.1016/j.anireprosci.2019.04.012)

Lima DBC, Silva LDMD, Marinari P & Comizzoli P 2020 Long-term preservation of testicular tissue integrity and viability using vitrification in the endangered black-footed ferret (*Mustela nigripes*). *Animals* **10** 1865. (https://doi.org/10.3390/ani10101865)

Lindau R, Vondra S, Spreckels J, Solders M, Svensson-Arvelund J, Berg G, Pollheimer J, Kaipe H, Jenmalm MC & Ernerudh J 2021 Decidual stromal cells support tolerance at the human foetal-maternal interface by inducing regulatory M2 macrophages and regulatory T-cells. *Journal of Reproductive Immunology* **146** 103330. (https://doi.org/10.1016/j.jri.2021.103330)

Liu C, Guo Y, Lu T, Li X, Guan W & Ma Y 2014 Establishment and genetic characteristics analysis of *in vitro* culture a fibroblast cell line derived from Wuzhishan miniature pig. *Cryobiology* **68** 281–287. (https://doi.org/10.1016/j.cryobiol.2014.02.009)

Liu T, Dou H, Xiang X, Li L, Li Y, Lin L, Pang X, Zhang Y, Chen Y, Luan J, *et al.* 2015 Factors determining the efficiency of porcine somatic cell nuclear transfer: data analysis with over 200,000 reconstructed embryos. *Cellular Reprogramming* **17** 463–471. (https://doi.org/10.1089/cell.2015.0037)

Liu Z, Cai Y, Wang Y, Nie Y, Zhang C, Xu Y, Zhang X, Lu Y, Wang Z, Poo M, *et al.* 2018 Cloning of macaque monkeys by somatic cell nuclear transfer. *Cell* **172** 881.e7–887.e7. (https://doi.org/10.1016/j.cell.2018.01.020)

Locatelli Y, Calais L, Duffard N, Lardic L, Monniaux D, Piver P, Mermillod P & Bertoldo MJ 2019 In vitro survival of follicles in prepubertal ewe ovarian cortex cryopreserved by slow freezing or non-equilibrium vitrification. *Journal of Assisted Reproduction and Genetics* **36** 1823–1835. (https://doi.org/10.1007/s10815-019-01532-8)

Loi P, Ptak G, Barboni B, Fulka Jr J, Cappai P & Clinton M 2001 Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. *Nature Biotechnology* **19** 962–964. (https://doi.org/10.1038/nbt1001-962)

Long CR, Westhusin ME & Golding MC 2014 Reshaping the transcriptional frontier: epigenetics and somatic cell nuclear transfer. *Molecular Reproduction and Development* **81** 183–193. (https://doi.org/10.1002/mrd.22271)

Lopes CA, Alves AM, Jewgenow K, Báo SN & de Figueiredo JR 2016 Cryopreservation of canine ovarian cortex using DMSO or 1,3-propanediol. *Theriogenology* **86** 1165–1174. (https://doi.org/10.1016/j.theriogenology.2016.04.006)

Lopes KRF, Praxedes ECG, Campos LB, Bezerra MB, Lima GL, Saraiva MVA & Silva AR 2018 Vitrification of ovarian tissue of Brazilian North-eastern donkeys (*Equus asinus*) using different cryoprotectants. *Reproduction in Domestic Animals* **53** 1060–1067. (https://doi.org/10.1111/rda.13203)

López A, Ducolomb Y, Casas E, Retana-Márquez S, Betancourt M & Casillas F 2021 Effects of porcine immature oocyte vitrification on actin microfilament distribution and chromatin integrity during early embryo development in vitro. *Frontiers in Cell and Developmental Biology* **9** 636765. (https://doi.org/10.3389/fcell.2021.636765)

Lu Y, West FD, Jordan BJ, Beckstead RB, Jordan ET & Stice SL 2015 Generation of avian induced pluripotent stem cells. *Methods in Molecular Biology* **1330** 89–99. (https://doi.org/10.1007/978-1-4939-2848-4_9)

Lucci CM, Kacinskis MA, Lopes LHR, Rumpf R & Baoa SN 2004 Effect of different cryoprotectants on the structural preservation of follicles in frozen zebu bovine (*Bos indicus*) ovarian tissue. *Theriogenology* **61** 1101–1114. (https://doi.org/10.1016/j.theriogenology.2003.06.004)

Lueders I, Luther I, Scheepers G & Van Der Horst G 2012 Improved semen collection method for wild felids: urethral catheterization yields high sperm quality in African lions (*Panthera leo*). *Theriogenology* **78** 696–701. (https://doi.org/10.1016/j.theriogenology.2012.02.026)

Lujić J, Marinović Z, Sušnik Bajec S, Djurdjevič I, Kása E, Urbányi B & Horváth Á 2017 First successful vitrification of salmonid ovarian tissue. *Cryobiology* **76** 154–157. (https://doi.org/10.1016/j.cryobiol.2017.04.005)

Luvoni GC & Pellizzari P 2000 Embryo development in vitro of cat oocytes cryopreserved at different maturation stages. *Theriogenology* **53** 1529–1540. (https://doi.org/10.1016/S0093-691X(00)00295-8)

Ma Y, Gu M, Chen L, Shen H, Pan Y, Pang Y, Miao S, Tong R, Huang H, Zhu Y, *et al.* 2021 Recent advances in critical nodes of embryo engineering technology. *Theranostics* **11** 7391–7424. (https://doi.org/10.7150/thno.58799)

Maclellan LJ, Carnevale EM, Coutinho Da Silva MA, Scoggin CF, Bruemmer JE & Squires EL 2002 Pregnancies from vitrified equine oocytes collected from super-stimulated and non-stimulated mares. *Theriogenology* **58** 911–919. (https://doi.org/10.1016/s0093-691x(02)00920-2)

Macpherson AJ, De Agüero MG & Ganal-Vonarburg SC 2017 How nutrition and the maternal microbiota shape the neonatal immune system. *Nature Reviews: Immunology* **17** 508–517. (https://doi.org/10.1038/nri.2017.58)

Magalhães LC, Bhat MH, Freitas JLS, Melo LM, Teixeira DIA, Pinto LCA, Câmara LMC, Duarte JMB & Freitas VJF 2017 The effects of cryopreservation on different passages of fibroblast cell culture in brown brocket deer (Mazama gouazoubira). Biopreservation and Biobanking15 463–468. (https://doi.org/10.1089/bio.2017.0060)

Manoli DS, Subramanyam D, Carey C, Sudin E, Van Westerhuyzen JA, Bales KL, Blelloch R & Shah NM 2012 Generation of induced pluripotent stem cells from the prairie vole. *PLoS ONE* **7** e38119. (https://doi.org/10.1371/journal.pone.0038119)

Marchetto MCN, Narvaiza I, Denli AM, Benner C, Lazzarini TA, Nathanson JL, Paquola ACM, Desai KN, Herai RH, Weitzman MD, *et al.* 2013 Differential L1 regulation in pluripotent stem cells of humans and apes. *Nature* **503** 525–529. (https://doi.org/10.1038/nature12686)

Martinez F 2017 Update on fertility preservation from the Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting: indications, results and future perspectives. *Human Reproduction* **32** 1802–1811. (https://doi.org/10.1093/humrep/dex218)

Martínez-Fresneda L, Castaño C, Bóveda P, Tesfaye D, Schellander K, Santiago-Moreno J & García-Vázquez FA 2019 Epididymal and ejaculated sperm differ on their response to the cryopreservation and capacitation processes in mouflon (Ovis musimon). *Scientific Reports* **9** 15659. (https://doi.org/10.1038/s41598-019-52057-0)

Martin-Wintle MS, Kersey DC, Wintle NJ, Aitken-Palmer C, Owen MA, Swaisgood RR. 2019 Comprehensive breeding techniques for the giant panda. In *Reproductive Sciences in Animal Conservation*. pp 275–308; Eds P Comizzoli, JL Brown & WV Holt Cham: Springer International Publishing.

Mastromonaco GF & Songsasen N 2020 Chapter 7 – Reproductive technologies for the conservation of wildlife and endangered species. In *Reproductive Technologies in Animals*. Ed **GA Presicce.** Academic Press.

Mestre-Citrinovitz AC, Sestelo AJ, Ceballos MB, Barañao JL & Saragüeta P 2016 Isolation of primary fibroblast culture from wildlife: the *Panthera onca* case to preserve a South American endangered species. *Current Protocols in Molecular Biology* **116** 28.7.1–28.7.14. (https://doi.org/10.1002/cpmb.25)

Mitchell RT & Williams SA 2022 A fertile future: fertility preservation special series. *Reproduction and Fertility* **3** C1–C3. (https://doi.org/10.1530/RAF-22-0001)

Mo X, Li N & Wu S 2014 Generation and characterization of bat-induced pluripotent stem cells. *Theriogenology* **82** 283–293. (https://doi.org/10.1016/j.theriogenology.2014.04.001)

Moawad AR, Zhu J, Choi I, Amarnath D, Chen W & Campbell KH 2013 Production of good-quality blastocyst embryos following IVF of ovine oocytes vitrified at the germinal vesicle stage using a cryoloop. *Reproduction, Fertility, and Development* **25** 1204–1215. (https://doi.org/10.1071/RD12215)

Montfort SL 2014. "Mayday Mayday Mayday", the Millennium Ark Is Sinking! In Reproductive Sciences in Animal Conservation, Advances in Experimental Medicine and Biology; W V Holt, JL Brown, P Comizzoli (eds.) 753, Springer Science+Business Media New York. pp 15–31. (https://doi.org/10.1007/978-1-4939-0820-2_2)

Mooney A 2021 The value of ex situ collections for global biodiversity conservation in the wild. *PhD Thesis*. Trinity College Dublin, School of Natural Sciences.

Moro LN, Hiriart MI, Buemo C, Jarazo J, Sestelo A, Veraguas D, Rodriguez-Alvarez L & Salamone DF 2015 Cheetah interspecific SCNT followed by embryo aggregation improves in vitro development but not pluripotent gene expression. *Reproduction* **150** 1–10. (https://doi.org/10.1530/REP-15-0048)

Morrow CJ, Penfold LM & Wolfe BA 2009 Artificial insemination in deer and non-domestic bovids. *Theriogenology* **71** 149–165. (https://doi.org/10.1016/j.theriogenology.2008.09.001)

Mouttham L & Comizzoli P 2016 The preservation of vital functions in cat ovarian tissues during vitrification depends more on the temperature of the cryoprotectant exposure than on the sucrose supplementation. *Cryobiology* **73** 187–195. (https://doi.org/10.1016/j.cryobiol.2016.07.013)

Mrowiec P, Bugno-Poniewierska M & Młodawska W 2021 The perspective of the incompatible of nucleus and mitochondria in interspecies somatic cell nuclear transfer for endangered species. *Reproduction in Domestic Animals* **56** 199–207. (https://doi.org/10.1111/rda.13864)

Na RS, Zhao QJ, Jin DP, Su XH, Chen XW, Guan WJ & Ma YH 2010 Establishment and biological characteristics of *Ujumqin* sheep fibroblast line. *Cytotechnology* **62** 43–52. (https://doi.org/10.1007/s10616-010-9260-6)

Nakayama K, Chinen S, Teshima J, Tamada Y, Hirabayashi M & Hochi S 2020 Silk fibroin sheet multilayer suitable for vitrification of in vitro-matured bovine oocytes. *Theriogenology* **145** 109–114. (https://doi.org/10.1016/j.theriogenology.2020.01.052)

Nowak A, Kochan J, Święţek E, Kij B, Prochowska S, Witarski W, Bugno-Poniewierska M & Niżański W 2020 Survivability and developmental competences of domestic cat (Felis catus) oocytes after Cryotech method vitrification. *Reproduction in Domestic Animals* **55** 992–997. (https://doi.org/10.1111/rda.13741)

O'Brien JK & Robeck TR 2006 Development of sperm sexing and associated assisted reproductive technology for sex preselection of captive bottlenose dolphins (*Tursiops truncatus*). *Reproduction, Fertility, and Development* **18** 319–329. (https://doi.org/10.1071/rd05108)

Oh HJ, Kim MK, Jang G, Kim HJ, Hong SG, Park JE, Park K, Park C, Sohn SH, Kim DY, *et al.* 2008 Cloning endangered gray wolves (Canis lupus) from somatic cells collected postmortem. *Theriogenology* **70** 638–647. (https://doi.org/10.1016/j.theriogenology.2008.04.032)

Okita K, Nagata N & Yamanaka S 2011 Immunogenicity of induced pluripotent stem cells. *Circulation Research* **109** 720–721. (https://doi.org/10.1161/RES.0b013e318232e187)

Okotrub KA, Mokrousova VI, Amstislavsky SY & Surovtsev NV 2018 Lipid droplet phase transition in freezing cat embryos and oocytes probed by Raman spectroscopy. *Biophysical Journal* **115** 577–587. (https://doi.org/10.1016/j.bpj.2018.06.019)

Ortiz-Escribano N, Bogado Pascottini O, Woelders H, Vandenberghe L, De Schauwer C, Govaere J, Van Den Abbeel E, Vullers T, Ververs C, Roels K, *et al.* 2018 An improved vitrification protocol for equine immature oocytes, resulting in a first live foal. *Equine Veterinary Journal* **50** 391–397. (https://doi.org/10.1111/evj.12747)

Otoi T, Yamamoto K, Koyama N & Suzuki T 1995 In vitro fertilization and development of immature and mature bovine oocytes cryopreserved by ethylene glycol with sucrose. *Cryobiology* **32** 455–460. (https://doi.org/10.1006/cryo.1995.1045)

Penfold LM & O'Brien JK 2012 Chapter 78 – Importation of nondomestic ruminant semen for management of zoological populations using artificial insemination. In: *Fowler's Zoo and Wild Animal Medicine: Current Therapy*, vol. 7, pp. 604–611. Eds Miller & **ME Fowler.** Elsevier Inc.

Penfold LM, Gillis JD, Pye GW & Pope EC 2021 Chapter 8 – Quality control practices and prevention of disease transmission in exotic and wild species. In *Manual of the International Embryo Technology Society*, 5th ed., pp. 8–13. International Embryo Technology Society.

Peng L, Zhou Y, Xu W, Jiang M, Li H, Long M, Liu W, Liu J, Zhao X & Xiao Y 2019 Generation of stable induced pluripotent stem-like cells from adult zebra fish fibroblasts. *International Journal of Biological Sciences* **15** 2340–2349. (https://doi.org/10.7150/ijbs.34010)

Peter RE, Lin HR & Van Der Kraak G 1988 Induced ovulation and spawning of cultured freshwater fish in China: advances in application of GnRH analogues and dopamine antagonists. *Aquaculture* **74** 1–10. (https://doi.org/10.1016/0044-8486(88)90080-4)

Pillai VV, Kei TG, Reddy SE, Das M, Abratte C, Cheong SH & Selvaraj V 2019 Induced pluripotent stem cell generation from bovine somatic cells indicates unmet needs for pluripotency sustenance. *Animal Science Journal* **90** 1149–1160. (https://doi.org/10.1111/asj.13272)

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM & Sexton JO 2014 The biodiversity of species and their rates of extinction, distribution, and protection. *Science* **344** 1246752. (https://doi.org/10.1126/science.1246752)

Poels J, Van Langendonckt A, Dehoux JP, Donnez J & Wyns C 2012 Vitrification of non-human primate immature testicular tissue allows maintenance of proliferating spermatogonial cells after xenografting to recipient mice. *Theriogenology* **77** 1008–1013. (https://doi.org/10.1016/j.theriogenology.2011.10.015)

Poo S & Hinkson KM 2019 Applying cryopreservation to anuran conservation biology. *Conservation Science and Practice* **1** e91. (https://doi.org/10.1111/csp2.91)

Pothana L, Makala H, Devi L, Varma VP & Goel S 2015 Germ cell differentiation in cryopreserved, immature, Indian spotted mouse deer (*Moschiola indica*) testes xenografted onto mice. *Theriogenology* **83** 625–633. (https://doi.org/10.1016/j.theriogenology.2014.10.028)

Pothana L, Venna NK, Devi L, Singh A, Chatterjee I & Goel S 2016 Cryopreservation of adult primate testes. *European Journal of Wildlife Research* **62** 619–626. (https://doi.org/10.1007/s10344-016-1024-y)

Pothana L, Devi L & Goel S 2017 Cryopreservation of adult cervid testes. *Cryobiology* **74** 103–109. (https://doi.org/10.1016/j.cryobiol.2016.11.008)

Praxedes ÉCG, Lima GL, Silva AM, Apolinário C, Bezerra JAB, Souza ALP, Oliveira MF, Rodrigues APR & Silva AR 2017 Characterisation and cryopreservation of the ovarian preantral follicle population from Spix's yellow-toothed cavies (Galea spixii Wagler, 1831). *Reproduction, Fertility and Development* **29** 594–602.

Prentice JR & Anzar M 2010 Cryopreservation of Mammalian oocyte for conservation of animal genetics. *Veterinary Medicine International* **2011** 146405. (https://doi.org/10.4061/2011/146405)

Pukazhenthi BS & Wildt DE 2004 Which reproductive technologies are most relevant to studying, managing and conserving wildlife? *Reproduction, Fertility, and Development* **16** 33–46. (https://doi.org/10.10371/RD03076)

Purdy PH 2006 A review on goat sperm cryopreservation. *Small Ruminant Research* **63** 215–225. (https://doi.org/10.1016/j.smallrumres.2005.02.015)

Purohit GN, Meena H & Solanki K 2012 Effects of vitrification on immature and in vitro matured, denuded and cumulus compact goat oocytes and their subsequent fertilization. *Journal of Reproduction and Infertility* **13** 53–59.

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-22-0005

Qu P, Shen C, Du Y, Qin H, Luo S, Fu S, Dong Y, Guo S, Hu F, Xue Y, *et al.* 2020 Melatonin protects rabbit somatic cell nuclear transfer (SCNT) embryos from electrofusion damage. *Scientific Reports* **10** 2186. (https://doi.org/10.1038/s41598-020-59161-6)

Quan GB, Wu GQ, Wang YJ, Ma Y, Lv CR & Hong QH 2016 Meiotic maturation and developmental capability of ovine oocytes at germinal vesicle stage following vitrification using different cryodevices. *Cryobiology* **72** 33–40. (https://doi.org/10.1016/j.cryobiol.2015.11.007)

Rall WF & Fahy GM 1985 Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. *Nature* **313** 573–575. (https://doi.org/10.1038/313573a0)

Ramaswamy K, Yik WY, Wang XM, Oliphant EN, Lu W, Shibata D, Ryder OA & Hacia JG 2015 Derivation of induced pluripotent stem cells from orangutan skin fibroblasts. *BMC Research Notes* **8** 577. (https://doi.org/10.1186/s13104-015-1567-0)

Reid CE, Hermes R, Blottner S, Goeritz F, Wibbelt G, Walzer C, Bryant BR, Portas TJ, Streich WJ & Hildebrandt TB 2009 Split-sample comparison of directional and liquid nitrogen vapour freezing method on post-thaw semen quality in white rhinoceroses (*Ceratotherium simum simum* and *Ceratotherium simum cottoni*). *Theriogenology* **71** 275–291. (https://doi.org/10.1016/j.theriogenology.2008.07.009)

Richer G, Baert Y & Goossens E 2020 In-vitro spermatogenesis through testis modelling: Toward the generation of testicular organoids. *Andrology* **8** 879–891. (https://doi.org/10.1111/andr.12741)

Rickard JP, Pool K, De Graaf SP, Portas T, Rourke N, Wiesner M, Hildebrandt TB, Göritz F & Hermes R 2022 Increasing the yield and cryosurvival of spermatozoa from rhinoceros ejaculates using the enzyme papain. *Biology* **11** 154. (https://doi.org/10.3390/biology11020154)

Rienzi L, Gracia C, Maggiulli R, Labarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S & Racowsky C 2017 Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Human Reproduction Update* **23** 139–155. (https://doi.org/10.1093/humupd/dmw038)

Rivers N, Daly J & Temple-Smith P 2020 New directions in assisted breeding techniques for fish conservation. *Reproduction, Fertility, and Development* **32** 807–821. (https://doi.org/10.1071/RD19457)

Robeck TR, Gearhart SA, Steinman KJ, Katsumata E, Loureiro JD & O'Brien JK 2011 In vitro sperm characterization and development of a sperm cryopreservation method using directional solidification in the killer whale (*Orcinus orca*). *Theriogenology* **76** 267–279. (https://doi.org/10.1016/j.theriogenology.2011.02.003)

Rodger JC 2019 Marsupials: progress and prospects. In *Reproductive Sciences in Animal Conservation*. Eds **P Comizzoli, JL Brown & WV Holt**. Cham: Springer International Publishing.

Rodrigues APR, Amorim CA, Costa SHF, Matos MHT, Santos RR, Lucci CM, Báo SN, Ohashi OM & Figueiredo JR 2004 Cryopreservation of caprine ovarian tissue using dimethylsulphoxide and propanediol. *Animal Reproduction Science* **84** 211–227. (https://doi.org/10.1016/j.anireprosci.2003.12.003)

Roth TL, Weiss RB, Buff JL, Bush LM, Wildt DE & Bush M 1998 Heterologous in vitro fertilization and sperm capacitation in an endangered African antelope, the scimitar-horned oryx (*Oryx dammah*). *Biology of Reproduction* **58** 475–482. (https://doi.org/10.1095/biolreprod58.2.475)

Roth TL, Stoops MA, Robeck TR & O'Brien JK 2016 Factors impacting the success of post-mortem sperm rescue in the rhinoceros. *Animal Reproduction Science* **167** 22–30. (https://doi.org/10.1016/j.anireprosci.2016.01.019)

Rutigliano HM, Thomas AJ, Umbaugh JJ, Wilhelm A, Sessions BR, Kaundal R, Duhan N, Hicks BA, Schlafer DH, White KL, *et al.* 2022 Increased expression of Pro-inflammatory cytokines at the fetal-maternal interface in bovine pregnancies produced by cloning. *American Journal of Reproductive Immunology* **87** e13520. (https://doi.org/10.1111/aji.13520)

Ryder OA & Onuma M 2018 Viable cell culture banking for biodiversity characterization and conservation. *Annual Review of Animal Biosciences* **6** 83–98. (https://doi.org/10.1146/annurev-animal-030117-014556)

Turathum B, Saikhun K, Sangsuwan P & Kitiyanant Y 2010 Effects of vitrification on nuclear maturation, ultrastructural changes and gene expression of canine oocytes. *Reproductive Biology and Endocrinology* **8** 70.

Sandler RL, Moses L & Wisely SM 2021 An ethical analysis of cloning for genetic rescue: case study of the black-footed ferret. *Biological Conservation* **257** 109118. (https://doi.org/10.1016/j.biocon.2021.109118)

Santymire R 2016 Implementing the use of a biobank in the endangered black-footed ferret (*Mustela nigripes*). *Reproduction, Fertility, and Development* **28** 1097–1104. (https://doi.org/10.1071/RD15461)

Saragusty J & Arav A 2011 Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. *Reproduction* **141** 1–19. (https://doi.org/10.1530/REP-10-0236)

Saragusty J, Gacitua H, King R & Arav A 2006 Post-mortem semen cryopreservation and characterization in two different endangered gazelle species (*Gazella gazella* and *Gazella dorcas*) and one subspecies (*Gazella gazelle acaiae*). *Theriogenology* **66** 775–784. (https://doi.org/10.1016/j.theriogenology.2006.01.055)

Saragusty J, Gacitua H, Pettit MT & Arav A 2007 Directional freezing of equine semen in large volumes. *Reproduction in Domestic Animals* **42** 610–615. (https://doi.org/10.1111/j.1439-0531.2006.00831.x)

Saragusty J, Hildebrandt TB, Behr B, Knieriem A, Kruse J & Hermes R 2009 Successful cryopreservation of Asian elephant (*Elephas maximus*) spermatozoa. *Animal Reproduction Science* **115** 255–266. (https://doi.org/10.1016/j.anireprosci.2008.11.010)

Saragusty J, Walzer C, Petit T, Stalder G, Horowitz I & Hermes R 2010 Cooling and freezing of epididymal sperm in the common hippopotamus (*Hippopotamus amphibius*). *Theriogenology* **74** 1256–1263. (https://doi.org/10.1016/j.theriogenology.2010.05.031)

Saragusty J, Ajmone-Marsan P, Sampino S & Modlinski JA 2020 Reproductive biotechnology and critically endangered species: merging in vitro gametogenesis with inner cell mass transfer. *Theriogenology* **155** 176–184. (https://doi.org/10.1016/j.theriogenology.2020.06.009)

Sato T, Katagiri K, Yokonishi T, Kubota Y, Inoue K, Ogonuki N, Matoba S, Ogura A & Ogawa T 2011 *In vitro* production of fertile sperm from murine spermatogonial stem cell lines. *Nature Communications* **2** 472. (https://doi.org/10.1038/ncomms1478)

Schmitt DL & Hildebrandt TB 1998 Manual collection and characterization of semen from Asian elephants (Elephas maximus). *Animal Reproduction Science* **53** 309–314. (https://doi.org/10.1016/s0378-4320(98)00120-1)

Seddon PJ & King M 2019 Creating proxies of extinct species: the bioethics of de-extinction. *Emerging Topics in Life Sciences* **3** 731–735. (https://doi.org/10.1042/ETLS20190109)

Segelbacher G, Bosse M, Burger P, Galbusera P, Godoy JA, Helsen P, Hvilsom C, Iacolina L, Kahric A, Manfrin C, *et al.* 2021 New developments in the field of genomic technologies and their relevance to conservation management. *Conservation Genetics* **23** 217–242. (https://doi.org/10.1007/s10592-021-01415-5)

Selvaraj V, Wildt DE & Pukazhenthi BS 2011 Induced pluripotent stem cells for conserving endangered species? *Nature Methods* **8** 805–807. (https://doi.org/10.1038/nmeth.1715)

Shapiro B 2017 Pathways to de-extinction: how close can we get to resurrection of an extinct species? *Functional Ecology* **31** 996–1002. (https://doi.org/10.1111/1365-2435.12705)

Sheets TP, Park CH, Park KE, Powell A, Donovan DM & Telugu BP 2016 Somatic cell nuclear transfer followed by CRIPSR/Cas9

microinjection results in highly efficient genome editing in cloned pigs. *International Journal of Molecular Sciences* **17** 2031. (https://doi.org/10.3390/ijms17122031)

Sherman JK 1963 Improved methods of preservation of human spermatozoa by freezing and freeze-drying. *Fertility and Sterility* **14** 49–64. (https://doi.org/10.1016/s0015-0282(16)34746-x)

Shi S, Tan Q, Liang J, Cao D, Wang S, Liang J, Chen K & Wang Z 2021 Placental trophoblast cell-derived exosomal microRNA-1290 promotes the interaction between endometrium and embryo by targeting LHX6. *Molecular Therapy: Nucleic Acids* **26** 760–772. (https://doi.org/10.1016/j.omtn.2021.09.009)

Si W, Lu Y, He X, Ji S, Niu Y, Tan T & Ji W 2010 116 Improved survival by cryopreserving rhesus macaque (*macaca mulatta*) spermatozoa with directional freezing technique. *Reproduction, Fertility and Development* **22** 217–217. (https://doi.org/10.1071/RDv22n1Ab116)

Silla AJ & Byrne PG 2019 The role of reproductive technologies in amphibian conservation breeding programs. *Annual Review of Animal Biosciences* **7** 499–519. (https://doi.org/10.1146/annurev-animal-020518-115056)

Silla AJ & Byrne PG 2021 Hormone-induced ovulation and artificial fertilisation in four terrestrial-breeding anurans. *Reproduction, Fertility, and Development* **33** 615 - 618. (https://doi.org/10.1071/RD20243)

Silla AJ, Calatayud NE & Trudeau VL 2021 Amphibian reproductive technologies: approaches and welfare considerations. *Conservation Physiology* **9** coab011. (https://doi.org/10.1093/conphys/coab011)

Silva AMD, Pereira AF, Comizzoli P & Silva AR 2020 Cryopreservation and culture of testicular tissues: an essential tool for biodiversity preservation. *Biopreservation and Biobanking* **18** 235–243. (https://doi.org/10.1089/bio.2020.0010)

Soulé M, Gilpin M, Conway W & Foose T 1985 The millenium ark: how long a voyage, how many staterooms, how many passengers? *Zoo biology* **5** 101–113. (https://doi.org/10.1002/zoo.1430050205)

Soulé ME (eds) 1987 *Viable Populations for Conservation*. Cambridge: Cambridge University Press. (https://doi.org/10.1017/CBO9780511623400)

Sowińska N, Zahmel J, Niżański W, Hribal R, Fernandez-Gonzalez L & Jewgenow K 2020 Meiotic status does not affect the vitrification effectiveness of domestic cat oocytes. *Animals* **10** 1371. (https://doi.org/10.3390/ani10081371)

Strand J 2021 *Biobanking as a Conservation Tool: The Way to an Amphibian Cell Line*. Ph.d.-serien for Det Ingeniør-og Naturvidenskabelige Fakultet, Aalborg Universitet.

Strand J, Thomsen H, Jensen JB, Marcussen C, Nicolajsen TB, Skriver MB, Sogaard IM, Ezaz T, Purup S, Callesen H, *et al.* 2020 Biobanking in amphibian and reptilian conservation and management: opportunities and challenges. *Conservation Genetics Resources* **12** 709–725. (https://doi.org/10.1007/s12686-020-01142-y)

Strand J, Callesen H, Pertoldi C & Purup S 2021 Establishing cell lines from fresh or cryopreserved tissue from the great crested newt (*Triturus cristatus*): a preliminary protocol. *Animals* **11** 367. (https://doi.org/10.3390/ani11020367)

Strauß S, Ziegler T, Allmeling C, Reimers K, Frank-Klein N, Seuntjens R & Vogt PM 2013 *In vitro* culture of skin cells from biopsies from the critically endangered Chinese giant salamander, *Andrias davidianus* (Blanchard, 1871) (Amphibia, Caudata, Cryptobranchidae). *Amphibian and Reptile Conservation* **5** 51–63.

Suzuki T, Boediono A, Takagi M, Saha S & Sumantri C 1996 Fertilization and development of frozen-thawed germinal vesicle bovine oocytes by a one-step dilution method in vitro. *Cryobiology* **33** 515–524. (https://doi.org/10.1006/cryo.1996.0055)

Swanson WF, Magarey GM & Herrick JR 2007 Sperm cryopreservation in endangered felids: developing linkage of in situ-ex situ populations. *Society of Reproduction and Fertility Supplement* **65** 417–432.

Sztein JM, Noble K, Farley JS & Mobraaten LE 2001 Comparison of permeating and nonpermeating cryoprotectants for mouse sperm cryopreservation. *Cryobiology* **42** 28–39. (https://doi.org/10.1006/cryo.2001.2300)

Taggart DA, Leigh CM, Steele VR, Breed WG, Temple-Smith PD & Phelan J 1996 Effect of cooling and cryopreservation on sperm motility and morphology of several species of marsupial. *Reproduction, Fertility, and Development* **8** 673–679. (https://doi.org/10.1071/rd9960673)

Takahashi K & Yamanaka S 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126** 663–676. (https://doi.org/10.1016/j.cell.2006.07.024)

Takeda K 2019 Functional consequences of mitochondrial mismatch in reconstituted embryos and offspring. *Journal of Reproduction and Development* **65** 485–489. (https://doi.org/10.1262/jrd.2019-089)

Tharasanit T & Thuwanut P 2021 Oocyte cryopreservation in domestic animals and humans: principles, techniques and updated outcomes. *Animals* **11** 2949. (https://doi.org/10.3390/ani11102949)

Tharasanit T, Buarpung S, Manee-In S, Thongkittidilok C, Tiptanavattana N, Comizzoli P & Techakumphu M 2012 Birth of kittens after the transfer of frozen–thawed embryos produced by intracytoplasmic sperm injection with spermatozoa collected from cryopreserved testicular tissue. *Reproduction in Domestic Animals* **47** (Supplement 6) 305–308. (https://doi.org/10.1111/rda.12072)

Thongphakdee A, Sukparangsi W, Comizzoli P & Chatdarong K 2020 Reproductive biology and biotechnologies in wild felids. *Theriogenology* **150** 360–373. (https://doi.org/10.1016/j.theriogenology.2020.02.004)

Thuwanut P, Srisuwatanasagul S, Wongbandue G, Tanpradit N, Thongpakdee A, Tongthainan D, Manee-In S & Chatdarong K 2013 Sperm quality and the morphology of cryopreserved testicular tissues recovered post-mortem from diverse wild species. *Cryobiology* **67** 244–247. (https://doi.org/10.1016/j.cryobiol.2013.07.002)

Toyooka Y, Tsunekawa N, Akasu R & Noce T 2003 Embryonic stem cells can form germ cells *in vitro*. *PNAS* **100** 11457–11462. (https://doi.org/10.1073/pnas.1932826100)

Traylor-Holzer K, Leus K & Byers O 2018 Integrating *ex situ* management options as part of a one plan approach to species conservation. In *The Ark and Beyond*. University of Chicago Press.

Turvey ST & Crees JJ 2019 Extinction in the Anthropocene. *Current Biology* **29** R982–R986. (https://doi.org/10.1016/j.cub.2019.07.040)

Unwin S & Pettit M 2004 Cryopreservation of Bennett's wallaby sperm using standard and directional cryopreservation techniques: preliminary results. In *AAZV AAWV WDA Joint Conference*, San Diego.

Uteshev V, Kaurova S, Shishova N, Stolyarov S, Browne R & Gakhova E 2015 In vitro fertilization with hormonally induced sperm and eggs from sharp-ribbed newts *Pleurodeles waltl*. *Russian Journal of Herpetology* **22** 35–40.

Vallorani C, Spinaci M, Bucci D, Porcu E, Tamanini C & Galeati G 2012 Pig oocyte vitrification by Cryotop method and the activation of the apoptotic cascade. *Animal Reproduction Science* **135** 68–74. (https://doi.org/10.1016/j.anireprosci.2012.08.020)

Van Den Berghe F, Paris DB, Van Soom A, Rijsselaere T, Van Der Weyde L, Bertschinger HJ & Paris MC 2012 Reproduction in the endangered African wild dog: basic physiology, reproductive suppression and possible benefits of artificial insemination. *Animal Reproduction Science* **133** 1–9. (https://doi.org/10.1016/j.anireprosci.2012.06.003)

Van Rooij P, Martel A, Haesebrouck F & Pasmans F 2015 Amphibian chytridiomycosis: a review with focus on fungus-host interactions. *Veterinary Research* **46** 137. (https://doi.org/10.1186/s13567-015-0266-0)

Vandevoort CA, Shirley CR, Hill DL & Leibo SP 2008 Effects of cryoprotectants and cryopreservation on germinal vesicle-stage

cumulus-oocyte complexes of rhesus monkeys. *Fertility and Sterility* **90** 805–816. (https://doi.org/10.1016/j.fertnstert.2007.06.105)

Vasconcelos GL, Saraiva MVA, Costa JJN, Passos MJ, Silva AWB, Rossi RODS, Portela AMLR, Duarte ABG, Magalhães-Padilha DM, Campelo CC, *et al.* 2013 Effects of growth differentiation factor-9 and FSH on in vitro development, viability and mRNA expression in bovine preantral follicles. *Reproduction, Fertility, and Development* **25** 1194–1203. (https://doi.org/10.1071/RD12173)

Veraguas D, Aguilera C, Echeverry D, Saez-Ruiz D, Castro FO & Rodriguez-Alvarez L 2020 Embryo aggregation allows the production of kodkod (*Leopardus guigna*) blastocysts after interspecific SCNT. *Theriogenology* **158** 148–157. (https://doi.org/10.1016/j.theriogenology.2020.09.006)

Verma R, Holland MK, Temple-Smith P & Verma PJ 2012 Inducing pluripotency in somatic cells from the snow leopard (*Panthera uncia*), an endangered felid. *Theriogenology* **77** 220–228, 228.e1–228.e2. (https://doi.org/10.1016/j.theriogenology.2011.09.022)

Verma R, Liu J, Holland MK, Temple-Smith P, Williamson M & Verma PJ 2013 Nanog is an essential factor for induction of pluripotency in somatic cells from endangered felids. *BioResearch Open Access* **2** 72–76. (https://doi.org/10.1089/biores.2012.0297)

Vieira AD, Forell F, Feltrin C & Rodrigues JL 2008 Calves born after direct transfer of vitrified bovine in vitro-produced blastocysts derived from vitrified immature oocytes. *Reproduction in Domestic Animals* **43** 314–318. (https://doi.org/10.1111/j.1439-0531.2007.00899.x)

Waggener WL & Carroll EJ 1998 A method for hormonal induction of sperm release in anurans (eight species) and *in vitro* fertilization in *Lepidobatrachus* species. *Development, Growth and Differentiation* **40** 19–25. (https://doi.org/10.1046/j.1440-169x.1998.t01-5-00003.x)

Wakayama T, Rodriguez I, Perry AC, Yanagimachi R & Mombaerts P 1999 Mice cloned from embryonic stem cells. *PNAS* **96** 14984–14989. (https://doi.org/10.1073/pnas.96.26.14984)

Walker CA, Bjarkadottir BD, Fatum M, Lane S & Williams SA 2021 Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation. *Human Fertility* **24** 188–198. (https://doi.org/10.1080/14647273.2019.1616118)

Wanderleya LS, Luza HKM, Faustino LR, Lima IMT, Lopes CAP, Silva AR, Báo SN, Campello CC, Rodrigues APR & de Figueiredo JR 2012 Ultrastructural features of agouti (*Dasyprocta aguti*) preantral follicles cryopreserved using dimethyl sulfoxide, ethylene glycol and propanediol. *Theriogenology* **77** 260–267. (https://doi.org/10.1016/j.theriogenology.2011.07.038)

Wani NA, Vettical BS & Hong SB 2017 First cloned Bactrian camel (*Camelus bactrianus*) calf produced by interspecies somatic cell nuclear transfer: a step towards preserving the critically endangered wild Bactrian camels. *PLoS ONE* **12** e0177800. (https://doi.org/10.1371/journal.pone.0177800)

Watt AM, Marcec-Greaves R, Hinkson KM, Poo S, Roberts B & Pitcher TE 2021 Effects of age on sperm quality metrics in endangered Mississippi gopher frogs (*Lithobates sevosus*) from captive populations used for controlled propagation and reintroduction efforts. *Zoo Biology* **40** 218–226. (https://doi.org/10.1002/zoo.21594)

Weeratunga P, Shahsavari A, Ovchinnikov DA, Wolvetang EJ & Whitworth DJ 2018 Induced pluripotent stem cells from a marsupial, the Tasmanian Devil (*Sarcophilus harrisii*): insight into the evolution of mammalian pluripotency. *Stem Cells and Development* **27** 112–122. (https://doi.org/10.1089/scd.2017.0224)

Whaley D, Damyar K, Witek RP, Mendoza A, Alexander M & Lakey JR 2021 Cryopreservation: an overview of principles and cell-specific considerations. *Cell Transplantation* **30** 963689721999617. (https://doi.org/10.1177/0963689721999617)

Whitworth DJ, Limnios IJ, Gauthier ME, Weeratunga P, Ovchinnikov DA, Baillie G, Grimmond SM, Graves JAM & Wolvetang EJ 2019 Platypus induced pluripotent stem cells: the unique pluripotency signature of a monotreme. *Stem Cells and Development* **28** 151–164. (https://doi.org/10.1089/scd.2018.0179)

WHO 2015 *Biodiversity and Health (Online)*. Biodiversity and Health. (available at: https://www.who.int/news-room/fact-sheets/detail/biodiversity-and-healthh) (who.int). Accessed on 14th July 2021.

Wiedemann C, Hribal R, Ringleb J, Bertelsen MF, Rasmusen K, Andersen CY, Kristensen SG & Jewgenow K 2012 Preservation of primordial follicles from lions by slow freezing and xenotransplantation of ovarian cortex into an immunodeficient mouse. *Reproduction in Domestic Animals* **47** (Supplement 6) 300–304. (https://doi.org/10.1111/rda.12081)

Wiedemann C, Zahmel J & Jewgenow K 2013 Short-term culture of ovarian cortex pieces to assess the cryopreservation outcome in wild felids for genome conservation. *BMC Veterinary Research* **9** 37. (https://doi.org/10.1186/1746-6148-9-37)

Wildt DE & Roth TL 1997 Assisted reproduction for managing and conserving threatened felids. *International Zoo Yearbook* **35** 164–172. (https://doi.org/10.1111/j.1748-1090.1997.tb01207.x)

Wildt DE, Comizzoli P, Pukazhenthi B & Songsasen N 2010 Lessons from biodiversity – the value of nontraditional species to advance reproductive science, conservation, and human health. *Molecular Reproduction and Development* **77** 397–409. (https://doi.org/10.1002/mrd.21137)

Wilmut I, Schnieke AE, Mcwhir J, Kind AJ & Campbell KH 1997 Viable offspring derived from fetal and adult mammalian cells. *Nature* **385** 810–813. (https://doi.org/10.1038/385810a0)

Woelders H, Windig J & Hiemstra SJ 2012 How developments in cryobiology, reproductive technologies and conservation genomics could shape gene banking strategies for (farm) animals. *Reproduction in Domestic Animals* **47** (Supplement 4) 264–273. (https://doi.org/10.1111/j.1439-0531.2012.02085.x)

Wolf DP & Hedrick JL 1971 A molecular approach to fertilisation. II. Viability and artificial fertilisation of *Xenopus laevis* gametes. *Developmental Biology* **25** 348–359. (https://doi.org/10.1016/0012-1606(71)90036-4)

Wu Z, Pan B, Qazi IH, Yang H, Guo S, Yang J, Zhang Y, Zeng C, Zhang M, Han H, *et al.* 2019 Melatonin improves in vitro development of vitrified-warmed mouse germinal vesicle oocytes potentially via modulation of spindle assembly checkpoint-related genes. *Cells* **8** 1009. (https://doi.org/10.3390/cells8091009)

Wunderlich S, Kircher M, Vieth B, Haase A, Merkert S, Beier J, Göhring G, Glage S, Schambach A, Curnow EC, *et al.* 2014 Primate iPS cells as tools for evolutionary analyses. *Stem Cell Research* **12** 622–629. (https://doi.org/10.1016/j.scr.2014.02.001)

Xin M, Siddique MAM, Dzyuba B, Cuevas-Uribe R, Shaliutina-Kolešová A & Linhart O 2017 Progress and challenges of fish sperm vitrification: a mini review. *Theriogenology* **98** 16–22. (https://doi.org/10.1016/j.theriogenology.2017.04.043)

Yamanaka S 2012 Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* **10** 678–684. (https://doi.org/10.1016/j.stem.2012.05.005)

Yang CY, Chen MC, Lee PT & Lin TT 2012 Cryopreservation of germinal vesicle stage porcine oocytes based on intracellular ice formation assessment. *Cryo Letters* **33** 349–362.

Zahmel J, Fernandez-Gonzalez L, Jewgenow K & Müller K 2019 Felid-gamete-rescue within EAZA-efforts and results in biobanking felid oocytes and sperm. *Journal of Zoo and Aquarium Research* **7** 15–24.

Zhang Y, Liu Y, Liu H & Tang WH 2019 Exosomes: biogenesis, biologic function and clinical potential. *Cell and Bioscience* **9** 19. (https://doi.org/10.1186/s13578-019-0282-2)

Zhou GB, Ma CB, Liu GS, Zhu SE, Zhang HH, Jia LL, Suo L, Shi JM, Wang YB, Tian TH, *et al.* 2009 Vitrification of farmed blue fox

oocytes in ethylene glycol and DMSO-based solutions using open-pulled straw (OPS). *Cryo Letters* **30** 112–118.

Zimkus BM, Hassapakis CL & Houck ML 2018 Integrating current methods for the preservation of amphibian genetic resources and viable tissues to achieve best practices for species conservation. *Amphibian and Reptile Conservation* **12** e165.

Zuo Y, Gao Y, Su G, Bai C, Wei Z, Liu K, Li Q, Bou S & Li G 2014 Irregular transcriptome reprogramming probably causes theca developmental failure of embryos produced by interspecies somatic cell nuclear transfer between the Przewalski's gazelle and the bovine. *BMC Genomics* **15** 1113. (https://doi.org/10.1186/1471-2164-15-1113)

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