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## [REVIEW]

# Strong Heterogeneity in Advances in Cryopreservation Techniques in the Mammalian Orders

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Between 1970 and 2012, vertebrate abundance has declined by 58% with an average annual decline of 2%, calling for serious action to prevent a mass extinction and an irreversible loss of biodiversity. Cryobanks and cryopreservation have the potential to assist and improve ex situ and in situ conservation strategies by storing valuable genetic material. A great deal of studies concerning cryopreservation have been performed within the class Mammalia, although no systematic overview has previously been presented. The objective of this study is therefore to evaluate the status, pattern and future of cryopreservation within Mammalia. A strong disproportional distribution of studies in examined orders is displayed. For the majority of examined orders less than 10% of species has been examined. However, the cryopreservation of germplasm has in several cases been successful and resulted in successful applications of assisted reproductive techniques (ARTs). Various obstacles are associated with the development of cryopreservation protocols, and among them the most prominent is interspecific differences in cryotolerance. Extrapolation of protocols in closely related species is considered the most applicable procedure, and a future supplement to overcome this problem is the examination and comparison of cryobiological traits. Successful protocols have been developed for the vast majority of domesticated mammals, which gives incentive for the further extrapolation of protocols in threatened species.

**Key words:** conservation, cryobanking, germplasm, assisted reproductive techniques, mammals

## INTRODUCTION

Biodiversity on earth is rapidly declining. The current rate of species extinction is unprecedented in human history and is already consistent with a mass extinction episode unmatched in the last 65 million years (Ceballos et al., 2015). Between 1970 and 2012, vertebrate abundance has declined by 58% with an average annual decline of 2% (WWF, 2016). The most common threat to declining populations is habitat loss and degradation (Rondinini et al., 2011; Heinrichs et al., 2016). This is evident for mammals living in terrestrial and freshwater habitats. However, the most common threat to marine mammals is overexploitation (WWF, 2016). Declines in population size reduce genetic diversity and increase the probability of inbreeding, leading to higher risk of extinction due to loss of adaptability and inbreeding depression (Wright et al., 2008; Hedrick and Garcia-Dorado, 2016). The afore-

mentioned threats are consequences of anthropogenic activity and we are therefore already finding ourselves in the middle of the Anthropocene epoch (Waters et al., 2016).

Due to the rapid loss of mammalian species, there is a desperate need for conservation strategies. The ideal solution is provided by in situ conservation, e.g., habitat preservation, however predictions of future exploitation of land make this strategy seemingly impossible (WWF, 2016). A less favorable approach is ex situ conservation, e.g., captive breeding programs. However, ex situ conservation should primarily be used as a complement to in situ conservation (Kasso and Balakrishnan, 2013). As an interface between these strategies, cryopreservation of biological material offers the opportunity to preserve endangered animals (Holt and Pickard, 1999). By storing cryopreserved gametes, embryos, or somatic cells, genetic diversity from existing wild or captive populations can be preserved (Johnston and Lacy, 1995; Leon-Quinto et al., 2009). To accommodate this, genome resource bank initiatives such as the Frozen Ark Consortium (<https://www.frozenark.org>) and Frozen Zoo (<http://institute.sandiegozoo.org/resources/frozen-zoo>) Con-

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tribute to the preservation of genetic material. Recovery of genetic material requires different extraction methods, depending on which material is to be preserved. These include, but are not limited to, electroejaculation (EE), manual stimulation (MS) or use of an artificial vagina (AV) for sperm, and post-mortem recovery of reproductive organs, e.g., epididymis or ovaries, for sperm or oocyte collection. Furthermore, optimal freezing methods and cryomedia are necessary. The most commonly applied freezing method is storage of the material in liquid nitrogen at  $-196^{\circ}\text{C}$  (Prieto et al., 2014). Chilling and freezing procedures often face problems with cold-shock stress, inflicting injuries and low quality rates in cryopreservation. To avoid this, the procedure often involves diluting the material in different media before chilling or freezing. The so-called extenders, such as egg yolk and antibiotics, are added to enrich and increase quality of the material. Cryoprotectants, such as glycerol, non-ionic sucrose, or lipoproteins, are added to prevent osmotic stress and intracellular ice formation (Fickel et al., 2007).

The susceptibility of biological material to injury during cryopreservation shows inter-specific differences, and optimal methods differ even between species that belong to the same phylogenetic group, e.g., order (Thurston et al., 2002). This requires examination of cryopreservation in virtually all (particularly endangered and unique) species to ensure the development of successful assisted reproductive techniques (ART). Aspects concerning cryopreservation of biological material have previously been outlined (Goodrowe et al., 2000; Mocé and Vicente, 2009; Rodger et al., 2009; Silva et al., 2016). However, a broad overview of Mammalia as a whole is lacking. The objective of this study is therefore to evaluate the status, pattern, and future of cryopreservation within the class Mammalia.

### THE STATUS OF CRYOPRESERVATION WITHIN MAMMALIA

In this review, the state of the art and the application of cryopreservation techniques in Mammalian species is presented. Motility has been emphasized for characterization of sperm quality and the development rate for characterization of embryo quality due to the prevalence of these parameters. Furthermore, every attempted use of ARTs has been included, regardless of success. Emphasis has been put on the attempt to present the progress of cryopreservation within each order by including all examined species. However, to represent the current progress of cryotechniques, few well-examined species have been thoroughly described. This review reserves its position on the inclusion of every cryopreservation study conducted to date.

### EMERGING PATTERNS OF CRYOPRESERVATION WITHIN MAMMALIA

A great deal of studies has been performed on species within Mammalia and has in several cases been successful and resulted in the successful application of ARTs (Table 1). Considering the vast number of species within this class, at least 2.7% of species has been examined, however further research is critically needed. For the majority of examined orders less than 10% of species has been examined, where some species have been subject to intense study and others have been subject to few. Further development of cryo-

preservation techniques could benefit from increased sharing of knowledge between researchers. This review serves as an overview of the class and as a preliminary foundation for the development of increased sharing of knowledge.

The most intensively examined species primarily consist of domesticated and captive wild animals. These protocols can be extrapolated to field conditions for wild animals to increase the genetic diversity of the current reserves of cryopreserved material within each species. This has already been accomplished in African elephant (*Loxodonta africana*) (Hildebrandt et al., 2012) and Japanese black bear (*Ursus thibetanus japonicus*) (Okano et al., 2006). Furthermore, a large proportion of examined species consists of non-threatened animals. Extrapolation of protocols from non-threatened to threatened species is another promising procedure, which have already been observed from common squirrel monkey (*Saimiri sciureus*) to black-headed squirrel monkey (*Saimiri vanzolinii*) (Oliveira et al., 2016) and generic grey wolf (*Canis lupus*) to Mexican grey wolf (*Canis lupus baileyi*) (Zindl et al., 2006).

### Extraction methods

Electroejaculation is the most prevalent extraction method of mammalian sperm, although this method has been observed to yield a lower sperm quality compared to other extraction methods. This has been observed in several species including domestic stallions (*Equus caballus*) (Cary et al., 2004) and grey wolf (Christensen et al., 2011). MS and AV in wild species require intensive animal training and conditioning, which has been successfully performed on captive whales (Robeck et al., 2010; Montano et al., 2012), monkeys (Takasu et al., 2016), and zebra (Crump and Crump, 1994). However, MS leads to other complications as seen in Asian elephant (*Elephas maximus*) where the mix of seminal plasma components can vary with each ejaculate and the risk of urine contamination is increased (Imrat et al., 2012). Due to poor results with the application of AV, MS, and EE in rhinos, a post-coital extraction method was applied as it includes the natural ejaculation of sperm. The small fluid volumes emitted by MS or EE may not consist of the appropriate mixture of seminal fluids needed to maintain sperm longevity and, ultimately, fertility (O'Brien and Roth, 2000b).

Another less invasive extraction method than EE is urethral catheterization which yielded superior motility figures for fresh sperm compared to EE in African lion (*Panthera leo*) (Lueders et al., 2012; Fernandez-Gonzales et al., 2015). The ejaculate volume was low, yet sperm motility was higher than sperm collected by EE and from cauda epididymes (Lueders et al., 2012). Therefore, urethral catheterization and post-coital extraction should be considered as alternative extraction methods in the future. In some species, female germplasm is extracted following euthanasia reducing the effective population size (Asada et al., 2000; Fujihira et al., 2006). However, oocytes can be collected surgically from live animals by follicular aspiration as seen in cynomolgus macaque (*Macaca fascicularis*) (Curnow et al., 2002) and vervet monkey (*Chlorocebus aethiops*) (Sparman et al., 2007).

When extracting germplasm from both males and females, reproduction seasonality should be taken into

account. Understanding the reproductive physiology of animals can contribute to optimizing extraction protocols (Santos et al., 2015). For example, tufted deer (*Elaphodus cephalophus*) sperm traits were observed to peak during autumn (Panyaboriban et al., 2016) and North American bison (*Bison bison*) sperm motility peaked during late summer and autumn (Krishnakumar et al., 2011). A similar tendency was indicated in Grant's zebra (*Equus quagga burchelli*), but was absent in the related Grevy's zebra (*Equus grevyi*) (Crump and Crump, 1994). Knowledge of the reproductive biology of each individual species is needed to enable optimal extraction.

### Freezing methods

The most frequently applied freezing method of the examined species is the conventional slow-freezing method, although other freezing methods have shown promising results. An alternative freezing method is vitrification, which has shown superior results in cryopreservation of testicular tissue from house mouse (Yokonishi et al., 2014) and blastocysts from house mouse (Yeoman et al., 2001). Vitrification offers the advantages of low cost, ease of operation, and the avoidance of extracellular ice formation (Rall and Fahy, 1985; Yeoman et al., 2001; Liu et al., 2009; Comizzoli et al., 2012). Several improvements to the vitrification method have been developed, to more efficiently vitrify biological material. These consist of ultra-rapid vitrification methods using smaller volumes and higher freezing rates, such as the cryotop method (Kuwayama, 2007) used for *Canis lupus baileyi* (Czarny et al., 2009) and *Sus scrofa domesticus* (Sakagami et al., 2010) oocytes. Freeze-drying is another alternative freezing method. Freezing of sperm by both slow-freezing and freeze-drying showed no significant difference in fertilization rates in rhesus macaque (Sánchez-Partida et al., 2008) and golden hamster (Muneto and Horiuchi, 2011). However, the freeze-drying method is convenient due as it does not require storage in liquid nitrogen, which makes it less expensive and well suited for long-term preservation combined with easier shipping at ambient temperature (Ward et al., 2003; Sánchez-Partida et al., 2008). Furthermore, the estimation of blastocyst development was calculated to have no significant decrease after fertilization with freeze-dried sperm kept at  $-80^{\circ}\text{C}$  for 100 years (Kawase et al., 2005). However, a downside of freeze-drying sperm is the immotility after rehydration, which excludes most ARTs, except intra cytoplasmic sperm injection (ICSI) (Sánchez-Partida et al., 2008; Muneto and Horiuchi, 2011).

An alternative to the conventional protocols for germplasm is the freezing of whole bodies. In Ogonuki et al. (2006), the successful fertilization of oocytes using ICSI was conducted with 15 year old sperm extracted from frozen whole bodies of house mouse kept at  $-20^{\circ}\text{C}$ . This investigation provides an incentive for further experiments using frozen whole bodies, which could simplify future cryopreservation methods. This may also enable de-extinction, as ARTs could be performed using animals preserved in permafrost (Ogonuki et al., 2006).

### Interspecific and intraspecific differences

The development of universal cryopreservation protocols is problematic as cryotolerance appears variate

between species (Holt, 2000; Thurston et al., 2002). Inter-specific variation was observed in closely related species after the application of identical cryopreservation protocols in rhinos (Portas et al., 2009) and squirrel monkeys (Oliveira et al., 2016). Moreover, sperm quality and cryotolerance have been observed to vary among individuals of the same species, which might relate to the genotype of the individual (Thurston et al., 2002; Gagliardi et al., 2008; Portas et al., 2009). This hypothesis is supported by the observation that cryotolerance did not differ within individual ejaculates from the same rhesus macaque (*Macaca mulatta*) (Gagliardi et al., 2008). Intraspecific sperm quality and cryotolerance have been found in Asian elephant. (Thongtip et al., 2004; Imrat et al., 2012) and white rhino (*Ceratotherium simum*) (Portas et al., 2009). Intraspecific differences are especially problematic, because not only must cryopreservation protocols be developed for the specific species, but it must also be tailored to suit the individual. If this is not taken into consideration, there is a possibility that cryopreservation protocols favor a specific genotype within each species. This is an unfavorable direction as it conflicts with the overall aim of cryobanking, which is to preserve as much genetic diversity as possible (Imrat et al., 2012).

### Transport and disease transmission risks

Cryobanking has demonstrated useful applications in ex situ conservation programs. The transport of frozen material is a less comprehensive procedure compared to the transport of live animals (Hermes et al., 2013; Saragusty et al., 2015). The application of frozen material in ex situ conservation programs was investigated in African elephant (Hildebrandt et al., 2012; Hermes et al., 2013). Cryopreserved sperm from wild African elephants were shipped from South Africa to Europe, where artificial insemination was performed on a captive female with the purpose of introducing new genes to the captive population. One pregnancy was successfully established (Hermes et al., 2013) and a later study reports the birth of two calves and one more pregnancy (Saragusty et al., 2015). These results are of great importance, as transport-induced stress in elephants increases the risk of mortality (Clubb et al., 2008). Furthermore, frozen epididymis and testis from house mouse (*Mus musculus*) were successfully shipped from the United Kingdom to Japan (Ogonuki et al., 2006). These successful endeavors are unique, because health legislation restricts the transport of genetic material across borders (Hermes et al., 2013; Saragusty et al., 2015). For the purpose of transportation, donors have to be investigated for a variety of pathogens, which excludes a lot of already cryopreserved material. In the successful transport of African elephant sperm, it was therefore important that a thorough clinical examination was performed on each donor, and blood samples were collected for disease screening at the time of collection (Hermes et al., 2013). Despite these efforts, cryopreservation protocols of male and female germplasm are not performed under sterile conditions (Bielanski et al., 2003). Furthermore, liquid nitrogen is not sterile as pathogenic organisms can be conserved on immersion. During transportation, these pathogenic organisms may be released back into the environment as nitrogen vapor cools dry shippers (Grout and Morris, 2009). These precautions should be



considered not only during transportation, but also at storage sites. Nitrogen vapor cools programmable freezers, which can release dormant pathogens to the surroundings (Grout and Morris, 2009) and contaminate samples, which are being prepared for cryopreservation or thawing. A problem arises when contaminated material is used in ARTs and thereby transferred to a live animal. However, it has been concluded that no direct evidence of disease transmission by transferred cryopreserved animal embryos have been seen in over 25 years (Bielanski, 2012).

### Implementation of cryopreservation

Cryobanking can work as a supporting tool for ex situ and in situ conservation programs (Leon-Quinto et al., 2009). However, which species should be prioritized is a matter for continuing discussion. It can reasonably be argued that focus should be on Critically Endangered (CR) listed species, as they might be on the brink of extinction. Cryopreservation of these species could work as a supplement to in situ conservation with the purpose of reversing the loss of heterozygosity in susceptible populations by introducing more genetically diverse material into the gene pool (Wildt, 2000).

The cryopreservation of threatened species could nevertheless face some obstacles. Firstly, inaccessibility of biological material and expenses related to the collection of this could prove to be an obstacle due to the small population size. Secondly, an increase in the genetic diversity of small populations could be insufficient as the selective pressure could be overwhelmed by the effects of genetic drift, resulting in no adaptive reaction to selective pressure (Pertoldi et al., 2007).

Further implementation of cryopreservation in ex situ and in situ conservation strategies could be prioritized, as transportation of cryopreserved material is more favorable than the transportation of live animals (Hildebrandt et al., 2009; Hermes et al., 2013). Cryopreserved germplasm could play a key role in continuous gene flow between captive and wild populations of the same species, effectively increasing the genetic diversity of ex situ populations and preserving the genetic diversity of the species as a whole. Cryopreserved germplasm and captive bred individuals conceived using cryopreserved germplasm, could then be reintroduced into the wild, increasing the population size sufficiently and reducing the effects of genetic drift (Holt and Pickard, 1999; Hermes et al., 2013). Alternatively, the future priority of cryopreservation could lie in the selection of species, which have a sufficient population size.

### The future of cryopreservation

In the future, efforts should be concentrated on the rather large gaps, particularly within the species-rich orders Rodentia and Chiroptera. This is especially relevant to Chiroptera spp., as to our knowledge no successful cryopreservation has been conducted within this order. Furthermore, focus is needed on the remaining Mammalia orders, which have not been examined at all.

The extraction of sperm post-coital or by urethral catheterization offers alternative extraction methods to the species, where prevalent extraction methods have been unsuccessful. These methods need further investigation in

other species to acknowledge their encouraging successes.

Promising and alternative freezing methods include vitrification and freeze-drying. However, these methods have not been implemented to the same extent as conventional freezing methods and further studies are needed to determine their application to different species. Also, little information is available of the long-term storage of freeze-dried sperm from other species than laboratory house mouse. Further research is needed on the possibility of storing freeze-dried sperm at a higher temperature than  $-80^{\circ}\text{C}$  for long periods of time (Kawase et al., 2005; Muneto and Horiuchi, 2011). Furthermore, estimations from Kawase et al. (2005) can be extrapolated to other cryopreservation protocols and thereby estimate the future success rates of freezing procedures.

Future experiments with the aim of simplifying freezing methods might also be an option. Both frozen and cooled sperm without cryoprotectants have shown successful results, which could give incentives to further protocols without cryoprotectants. Furthermore, the success of ICSI using sperm from a frozen whole mouse (Ogonuki et al., 2006) may encourage zoological gardens worldwide to store deceased animals in an ordinary deep freezer, when equipment for standard cryopreservation methods is unavailable.

Protocols developed for laboratory conditions serve as important groundwork for the development of protocols for field conditions. Protocol adjustments have to be made when extracting and handling biological material from wild populations, as field conditions rarely provide sufficient equipment for proper cryopreservation.

Examination of cryobiological traits prior to cryopreservation could be performed, as optimal protocols depend on these traits. Application of methods previously deemed successful for a particular set of traits could prove to be the optimal foundation when working with non-examined species. This has the potential of overcoming the difficulties associated with interspecific differences in cryotolerance (Comizzoli et al., 2012).

In the future, it will be necessary to exercise precaution against the risk of contamination; sterilization of liquid nitrogen by UV irradiation (Parmegiani et al., 2010) and disinfection of storage units (Bielanski, 2012) should therefore be implemented in cryopreservation protocols. Recommended methods and procedures to diminish the risk of disease transmission from post-thaw embryos and sperm to live animals is summarized in Bielanski (2012). Additionally, a thorough health examination of the donor animals could be considered to increase the chances of a later approval of transport across borders.

It has recently been suggested that the microbiome of animals may have implications for the successful reintroduction of animal species into the wild (Bahndorff et al., 2016). Consequently, it could be argued that characterization of the microbiome and development of protocols for cryopreservation of symbiotic microorganisms should be considered, when developing cryopreservation protocol for a species of conservation interest.

To augment the overview of cryopreservation in Mammalia beyond the accomplishments of this review, the development of a peer-reviewed online database could be considered as it offers an easy and accessible overview,

**Table 1.** Species and subspecies included in this study in which the effect of cryopreservation on biological material has been examined. 'Cryopreservation' refers to the commonly used slow freezing method. Deviations around the mean are presented as standard error of mean, unless followed by \* in which case it presents the standard deviation. \*\* Denotes range of values.

Family	Species	Material	Extraction method	Preservation method	Result	Reference
<b>Order: Carnivora</b>						
Canidae	<i>Canis latrans</i>	Sperm	Electroejaculation	Cryopreservation	Up to 57.5% progressive motility post-thaw	Minter and Deliberto., 2005
	<i>Canis lupus baileyi</i>	Sperm	Electroejaculation	Cryopreservation	~65.5% progressive motility post-thaw when cooled 2.5 hours before freezing	Zindl et al., 2006
		Oocytes	Aspiration from ovaries	Vitrification	57.1% live intact oocytes post-thaw	Czarny et al., 2009
	<i>Canis lupus familiaris</i>	Sperm	Manual stimulation	Cryopreservation	65.83 ± 4.7% motility post-thaw when sample is diluted with coconut extender before freezing	Cardoso et al., 2006
		Sperm	Manual stimulation	Cryopreservation	41.2 ± 1.6% progressive motility post-thaw when thawed in 70°C water bath	Nöthling and Shuttleworth, 2005
		Sperm	Manual stimulation	With ultra-freezer (without liquid nitrogen)	70.38 ± 0.94% motility post-thaw	Álamo et al., 2005
		Sperm	Manual stimulation	Cryopreservation	70.9 ± 2.5% motility post-thaw. Artificial insemination was successful and resulted in a whelping rate of 57.1%	Nizanski, 2006
		Embryos	Uterine flushing	Slow freezing	Viability rate of 66.5% following in vitro culture post-thaw	Guaitolini et al., 2012
	<i>Canis lupus rufus</i>	Sperm	Electroejaculation	Cryopreservation	~46% motility post-thaw	Lockyear et al., 2009
	<i>Chrysocyon brachyurus</i>	Sperm	Electroejaculation	Cryopreservation	20.0 ± 1.9% motility post-thaw	Johnson et al., 2014
	<i>Vulpes vulpes</i>	Sperm	Manual stimulation	Cryopreservation	65 ± 0.4 progressive motility post-thaw. Artificial insemination was successful and resulted in an overall conception rate of 75% and 6 pups per litter	Farstad et al., 1992
Felidae	<i>Acinonyx jubatus</i>	Sperm	Electroejaculation	Cryopreservation	Sperm motility index of 62.9 ± 4.7 when centrifuged through an Accudenz gradient post-thaw	Crosier et al., 2009
	<i>Felis catus</i>	Sperm	Electroejaculation	Cryopreservation	71.8 ± 11.0% progressive motility post-thaw	Klaus et al., 2016
		Oocytes	Ovariohysterectomy	Vitrification	71.5% survival rate of partially delipidated oocytes post-thaw. In vitro fertilization resulted in the birth of 1 live kitten	Galiguis et al., 2014
		Oocytes	Ovariohysterectomy	Vitrification	50.2% survival rate post-thaw	Merlo et al., 2008
	<i>Leopardus pardalis</i>	Sperm	Electroejaculation	Cryopreservation	Sperm motility index of up to 50.0 ± 1.8 post-thaw	Baudi et al., 2008
	<i>Leopardus tigrinus</i>	Sperm	Electroejaculation	Cryopreservation	Sperm motility index of up to 51 ± 4 post-thaw	Baudi et al., 2008
	<i>Lynx pardinus</i>	Sperm	Electroejaculation	Cryopreservation	~34% motility post-thaw. Fertilized heterologous oocytes had a cleavage rate of 44.7 ± 19.6%	Gañán et al., 2009a
	<i>Lynx rufus</i>	Sperm	Electroejaculation	Cryopreservation	~50% motility post-thaw. 27% of fertilized heterologous oocytes reached the morula stage	Gañán et al., 2009b
	<i>Neofelis nebulosa</i>	Sperm	Electroejaculation	Cryopreservation	35.0 ± 3.6% motility post-thaw	Pukazhenthi et al., 2006

	<i>Panthera leo</i>	Sperm	Electroejaculation	Cryopreservation	~10% motility post-thaw. Development of blastocysts following in vitro maturation and intracytoplasmic sperm injection of oocytes post-thaw was achieved	Fernandez-Gonzalez et al., 2015
	<i>Prionailurus viverrinus</i>	Sperm	Electroejaculation	Cryopreservation	68% motility post-thaw. Fertilized heterologous cat oocytes had a cleavage rate of 62.1%	Thiangtum et al., 2006
Ursidae	<i>Ailuropoda melanoleuca</i>	Sperm	Electroejaculation	Cryopreservation	~58% motility after 1. freezing cycle. ~51% motility after 2. freezing cycle	Santiago-Moreno et al., 2016
		Sperm	Electroejaculation	Cryopreservation	70.75 ± 2.0% motility post-thaw. Live cubs were born with a birth rate of up to 75% following artificial insemination	Huang et al., 2012
	<i>Ursus arctos</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	23.7 ± 2.3% motility post-thaw	Anel et al., 2011
	<i>Ursus thibetanus japonicus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 20% motility post-thaw	Okano et al., 2004
		Sperm	Electroejaculation	Cryopreservation	36.3 ± 5.1% motility post-thaw	Okano et al., 2006
Order: Cetartiodactyla						
Balaenopteridae	<i>Balaenoptera acutorostrata</i>	Sperm	Recovery of sperm from vasa deferentia post-mortem	Cryopreservation	Up to 20% motility post-thaw	Mogoe et al., 1998
		Oocytes	Recovery of follicular oocytes post-mortem	Cryopreservation	Morphological viability of 39.7% post-thaw. Up to 30% of cryopreserved follicular oocytes resumed meiosis during in vitro maturation.	Asada et al., 2000
	<i>Balaenoptera bonaerensis</i>	Oocytes	Recovery of oocytes post-mortem	Vitrification	Up to 46.2% of matured oocytes cleaved following in vitro maturation and intra cytoplasmic sperm injection post-thaw.	Fujhara et al., 2006
	<i>Balaenoptera edeni</i>	Sperm	Recovery of sperm from vasa deferentia post-mortem	Cryopreservation	Regardless of motility and viability more than 90% of mouse oocytes were activated following intra cytoplasmic sperm injection using frozen-thawed sperm.	Watanabe et al., 2007
Bovidae	<i>Aepyceros melampus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 70% motility and penetration of 64% of oocytes with frozen-thawed sperm following IVF	Rush et al., 1997
	<i>Ammotragus lervia sahariensis</i>	Sperm	Ultrasound-guided massage and electroejaculation	Cryopreservation	Up to 29.5 ± 6.5% motility post-thaw	Santiago-Moreno et al., 2013
	<i>Antidorcas marsupialis</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	~65% total motility post-thaw	Chatiza et al., 2012
	<i>Antilope cervicapra</i>	Sperm	Electroejaculation	Cryopreservation	~40% motility post-thaw. Artificial insemination using frozen-thawed sperm resulted in birth of a live individual	Holt et al., 1988
	<i>Bison bison bison</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 39.0 ± 8.2% progressive motility post-thaw. Up to 21.6 ± 11.0% of bovine oocytes developed into blastocysts following heterologous in vitro fertilization using frozen-thawed sperm.	Krishnakumar et al., 2011

<i>Bison bison athabasca</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 12.5 ± 9.2% progressive motility post-thaw. Up to 22.3 ± 13.5% of bovine oocytes developed into blastocysts following heterologous in vitro fertilization using frozen-thawed sperm.	Krishnakumar et al., 2011
<i>Bison bonasus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 44.3 ± 14.0% motility post-thaw and a penetration of 63.1 ± 15.9% of bovine oocytes following in vitro fertilization	Pérez-Garnelo et al., 2006
<i>Bos frontalis</i>	Sperm	Rectal massage	Cryopreservation	Up to 40.6 ± 1.7% progressive motility post-thaw	Baruah et al., 2012
<i>Bos grunniens</i>	Oocytes	Recovery of oocytes post-mortem	Vitrification	Up to 9% of fertilized oocytes developed into blastocysts following in vitro fertilization using <i>B. grunniens</i> sperm	Niu et al., 2014
<i>Bos javanicus</i>	Sperm	Electroejaculation, rectal massage and a combination of these	Cryopreservation	56.7 ± 0.7% progressive motility using rectal massage	Sarsaifi et al., 2013
<i>Bos taurus</i>	Embryos	Recovery of oocytes followed by in vitro fertilization	Vitrification	Up to 43.5% pregnancy rate and no embryo loss after 60 days following embryo transfer of frozen-thawed embryos	Pereira et al., 2016
	Oocytes	Recovery of oocytes post-mortem	Vitrification	Up to 1% of embryos expanded following in vitro fertilization using frozen-thawed partially denuded oocytes, compared to 0% for intact cumulus oocyte complexes	Ševelová and Lopatářová, 2012
	Sperm	Recovery of sperm from vasa deferentia and epididymis	Cryopreservation	Up to 51.9% motility post-thaw	Strand et al., 2016
<i>Bubalis bubalis</i>	Sperm	Artificial vagina	Cryopreservation	Up to 48.3 ± 1.7% motility post-thaw.	Reddy et al., 2010
<i>Capra falconeri heptneri</i>	Sperm	Electroejaculation	Cryopreservation	Up to 41.5 ± 5.6% total motility post-thaw	Bezjian et al., 2013
<i>Capra hircus ancyrensis</i>	Sperm	Artificial vagina	Cryopreservation	Up to 17.0 ± 3.8% progressive motility post-thaw.	Bucak et al., 2010
<i>Capra pyrenaica</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 79.2 ± 5.6 motility post-thaw	Fernández-Santos et al., 2011
<i>Capricornis crispus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 11.8 ± 4.1% motility post-thaw.	Kashiwazaki et al., 2001
<i>Capricornis sumatraensis</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	Up to 58.6% DNA integrity post-thaw	Thuwanut et al., 2013
<i>Connochaetes taurinus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 62.5 ± 3.0% motility post-thaw	Schiewe et al., 1991
<i>Damaliscus pygargus phillipsi</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	~85% total motility post-thaw	Chatiza et al., 2012
<i>Gazella dorcas</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	21.0 ± 4.6% progressive motility post-thaw	Saragusty et al., 2006
<i>Gazella gazella gazella</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	Up to 15.3 ± 2.7% progressive motility post-thaw	Saragusty et al., 2006
<i>Gazella gazella acaicae</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	2.33 ± 0.3% progressive motility post-thaw	Saragusty et al., 2006



	<i>Kobus ellipsiprymnus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 72% progressive motility post-thaw	Watson et al., 1997
	<i>Litocranius walleri walleri</i>	Sperm	Electroejaculation	Cryopreservation	26 ± 8% motility post-thaw. No living offspring resulted from artificial insemination using frozen-thawed sperm	Penfold et al., 2005
	<i>Oreotragus oreotragus</i>	Oviductal cells	Recovery of oviducts post-mortem	Cryopreservation	A four-cell embryo developed following in vitro fertilization using <i>O. oreotragus</i> sperm and oocytes. The fertilized oocytes were cocultured with frozen-thawed oviductal cells.	Raphael et al., 1991
	<i>Oryx dammah</i>	Sperm	Electroejaculation	Cryopreservation	~78% motility post-thaw. Following IVF with domestic cow oocytes, cleaving occurred in up to 72.7% of inseminated oocytes	O'Brien and Roth, 2000a
	<i>Ovis aries</i>	Sperm	Artificial vagina	Cryopreservation	Artificial insemination of female <i>O. aries</i> using frozen-thawed sperm yielded up to a 73.3% pregnancy rate	Khalifa et al., 2013
	<i>Ovis orientalis musimon</i>	Sperm	Electroejaculation	Cryopreservation	20% motility post-taw	Morar et al., 2010
	<i>Rupicapra pyrenaica</i>	Sperm	Ultrasonic-guided massage	Cryopreservation	Up to 47.5 ± 2.1% motility post-thaw	Pradiee et al., 2016
	<i>Syncerus caffer</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 14 ± 13% progressive motility post-thaw	Herold et al., 2006
	<i>Taurotragus oryx</i>	Sperm	Electroejaculation	Cryopreservation	Best result of sperm motility and morphology post-thaw was the Bettsville with glycerol fraction added after cooling rate of 4°C	Lozano et al., 2016
	<i>Tetracerus quadricornis</i>	Oocytes	Recovery of oocytes post-mortem	Vitrification	Following IVM of frozen-thawed oocytes, 11.7 ± 2.2% reached metaphase II	Rao et al., 2011
	<i>Tragelaphus erycerus</i>	Sperm	Electroejaculation	Cryopreservation	Best result of sperm motility and morphology post-thaw was the Bettsville with glycerol fraction added at room temperature	Lozano et al., 2016
	<i>Tragelaphus strepsiceros</i>	Sperm	Electroejaculation	Cryopreservation	50.8 ± 2.7% motility post-thaw	Schiewe et al., 1991
Camelidae	<i>Camelus bactrianus</i>	Sperm	Artificial vagina	Cryopreservation	Up to 29.9% progressive motility post-thaw	Niasari-Naslaji et al., 2007
	<i>Camelus dromedarius</i>	Sperm	Artificial vagina	Cryopreservation	61.6 ± 4.6% motility post-thaw	Bahrawy et al., 2012
	<i>Lama glama</i>	Sperm	Electroejaculation	Cryopreservation	22.5 ± 4.4% motility post-thaw	Carretero et al., 2015
	<i>Lama guanicoe</i>	Sperm	N/A	Cold storage of testicles	No motile sperm	Maksudov et al., 2008
	<i>Vicugna pacos</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	21.5 ± 3.5% motility post-thaw	Morton et al., 2010
Cervidae	<i>Alces alces</i>	Sperm	Recovery of sperm from vasa deferentia, cauda epididymis, and ampullae post-mortem	Cryopreservation	Up to 20% motility post-thaw	Krzywiński, 1981
	<i>Axis axis</i>	Sperm	Electroejaculation	Cryopreservation	Up to 69 ± 2.4% motility post-thaw	Haigh et al., 1993
	<i>Capreolus capreolus</i>	Sperm	Electroejaculation	Cryopreservation	39.5 ± 5.2% motility post-thaw	Prieto-Pablos et al., 2016

	<i>Cervus elaphus hispanicus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	60.2 ± 3.2% motility post-thaw	Anel-López et al., 2012
	<i>Cervus nippon taiounua</i>	Sperm	Electroejaculation	Cryopreservation	73 ± 3% vigorous motility post-thaw	Cheng et al., 2004
	<i>Dama dama</i>	Embryo	Laparoscopic recovery of embryos	Cryopreservation	Embryo transfer yielded 26% pregnancy rate post-thaw	Morrow et al., 1994
	<i>Elaphodus cephalophus</i>	Sperm	Electroejaculation	Cryopreservation	53.9 ± 7.4% motility post-thaw	Panyaboriban et al., 2016
	<i>Muntiacus feae</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	35% DNA integrity post-thaw	Thuwanut et al., 2013
	<i>Odocoileus virginianus</i>	Sperm	Electroejaculation	Cryopreservation	~60% motility post-thaw	Stewart et al., 2016
	<i>Rucervus eldii siamensis</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 30% motility post-thaw	Thuwanut et al., 2012
	<i>Rusa timorensis</i>	Sperm	Electroejaculation	Cryopreservation	52.50 ± 5.9%* motility post-thaw	Nalley et al., 2011
	<i>Rusa unicolor swinhoei</i>	Sperm	Electroejaculation	Cryopreservation	74 ± 3% vigorous motility post-thaw	Cheng et al., 2004
Delphinidae	<i>Lagenorhynchus obliquidens</i>	Sperm	Manual stimulation	Directional freezing and cryopreservation	Directional freezing yielded 94% motility post-thaw compared to 48% post-thaw motility following cryopreservation. 33.3% live births following artificial insemination using frozen-thawed sperm.	Robeck et al., 2009
	<i>Tursiops truncatus</i>	Sperm	Manual stimulation	Cryopreservation	66.7 ± 0.6% motility post-thaw	Montano et al., 2012
		Sperm	Manual stimulation	Cryopreservation	60% progressive motility post-thaw. 50% live births following artificial insemination using frozen-thawed sperm	O'Brien and Robeck, 2006
	<i>Orcinus orca</i>	Sperm	Manual stimulation	Cryopreservation	54.1 ± 1.3% motility post-thaw	Robeck et al., 2011
Giraffidae	<i>Giraffa camelopardalis</i>	Sperm	Natural ejaculation	Freeze-drying	Following intra cytoplasmic sperm injection, 50% of oocytes survived and formed pronuclei	Kaneko et al., 2014
	<i>Okapia johnstoni</i>	Sperm	Electroejaculation	N/A	Intolerance to osmotic pressure was observed, therefore material was not frozen. Tolerance of cooling without additives was observed however	Penfold, 2008
Hippopotamidae	<i>Choeropsis liberiensis</i>	Sperm	Electroejaculation	Cooling	51.0 ± 16.5% motility after chilling	Saragusty et al., 2010a
	<i>Hippopotamus amphibius</i>	Sperm	Recovery of sperm from epididymis following castration	Cryopreservation	18.86 ± 8.0% motility post-thaw	Saragusty et al., 2010b
Monodontidae	<i>Delphinapterus leucas</i>	Sperm	Manual stimulation	Cryopreservation	40.5 ± 0.8% motility post-thaw. Birth of two calves of which one survived following artificial insemination where 20% of cases resulted in pregnancy, using frozen-thawed sperm	Robeck et al., 2010
		Sperm	Combined manual stimulation and artificial vagina	Directional freezing	18.2 ± 0.3% progressive motility post-thaw	O'Brien and Robeck, 2010
Suidae	<i>Phacocoerus aethiopicus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 35% progressive motility post-thaw	Watson et al., 1997
	<i>Sus scrofa</i>	Sperm	Manual stimulation	Cryopreservation	44.3 ± 7.7% motility post-thaw	Shiomi et al., 2015
	<i>Sus scrofa domesticus</i>	Embryos	Surgically	Vitrification	Up to 84.4% survival rate of expanding blastocytes post-thaw	Fujino et al., 2008

		Sperm	Gloved-hand method	Cryopreservation	Up to 65.55% total motility post-thaw	Mercado et al., 2009
		Embryos	Surgically	Vitrification	Up to 79% viability post-thaw	Sakagami et al., 2010
Tayassuidae	<i>Pecari tajacu</i>	Sperm	Electroejaculation	Cryopreservation	46.4 ± 3.9% total motility post-thaw. Aloe vera provided an alternative supplement to cryodiluents	Souza et al., 2016
Tragulidae	<i>Moschiola indica</i>	Sperm	Collection of testis post-mortem	Cryopreservation	Xenografting of frozen-thawed testis onto mice led to the production of sperm	Pothana et al., 2015
<b>Order: Chiroptera</b>						
Pteropodidae	<i>Pteropus poliocephalus</i>	Sperm	Electroejaculation	Cooling	~40% motility	de Jong et al., 2005
	<i>Pteropus giganteus</i>	Sperm	Electroejaculation	Cooling	Displayed cold shock injury with ~5% intact acrosomes	Melville et al., 2012
	<i>Pteropus hypomelanus</i>	Sperm	Electroejaculation	Cooling	Displayed cold shock injury with ~20% intact acrosomes	Melville et al., 2012
	<i>Pteropus vampyrus</i>	Sperm	Electroejaculation	Cooling	Displayed cold shock injury with ~30% intact acrosomes	Melville et al., 2012
<b>Order: Dasyuromorphia</b>						
Dasyuridae	<i>Dasyurus hallucatus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	No motility or viability post-thaw, but fresh sperm showed nontoxic levels of glycerol up to 40%	Czarny et al., 2009
	<i>Dasyurus viverrinus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	No motility or viability post-thaw, but fresh sperm showed nontoxic levels of glycerol up to 40%	Czarny et al., 2009
	<i>Sminthopsis crassicaudata</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	No motility or viability post-thaw, but fresh sperm showed nontoxic levels of glycerol up to 40%	Czarny et al., 2009
	<i>Sarcophilus harrisii</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	48 ± 1.7% viability post-thaw	Keeley et al., 2012
<b>Order: Diprotodontia</b>						
Macropodidae	<i>Macropus eugenii</i>	Sperm	Electroejaculation	Cryopreservation	10 ± 2% motility post-thaw	Molinia and Rodger, 1996
	<i>Macropus giganteus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	~12-14% motility when using DMA cryodiluent post-thaw which was better than glycerol	McClean et al., 2008
Phalangeridae	<i>Trichosurus vulpecula</i>	Sperm	Electroejaculation	Cryopreservation	68 ± 3.2% motility post-thaw	Molinia and Rodger, 1996
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	82% motility post-thaw	Taggart et al., 1996
Phascolarctidae	<i>Phascolarctos cinereus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 50% (35-65)** motility post-thaw	Johnston et al., 2006
		Sperm	Electroejaculation	Cooling	Artificial insemination has been successful using cooled sperm	Allen et al., 2008
Potoridae	<i>Potorous longipes</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	6% motility post-thaw	Taggart et al., 1996
Pseudocheiridae	<i>Pseudocheirus peregrinus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	23% motility post-thaw	Taggart et al., 1996
Vombatidae	<i>Vombatus ursinus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 84% (71-94)** motility post-thaw	Johnston et al., 2006
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	~75% motility post-thaw	MacCallum and Johnston, 2005

		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	~45% viability post-thaw	Bickell et al., 2001
<b>Order: Lagomorpha</b>						
Leporidae	<i>Lepus europaeus</i>	Sperm	Electroejaculation	Cryopreservation	Up to 46.9 ± 5.8% motility post-thaw.	Hildebrandt et al., 2009
	<i>Oryctolagus cuniculus</i>	Sperm	Artificial vagina	Double cryopreservation	Up to 18.1 ± 2.2% motility post-thaw. Fertility and kindling rate after first freezing-cycle were up to 73.9% and 35.7% after second cycle	Si et al., 2006
<b>Order: Monotremata</b>						
Tachyglossidae	<i>Tachyglossus aculeatus</i>	Sperm	Recovery of sperm from epididymis post-mortem and manual stimulation	Cryopreservation	Average membrane integrity of ~30% post-thaw	Johnston et al., 2009
<b>Order: Peramelemorphia</b>						
Peramelidae	<i>Isodon macrourus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	45% motility post-thaw	Taggart et al., 1996
<b>Order: Perissodactyla</b>						
Equidae	<i>Equus asinus</i>	Sperm	Artificial vagina	Cryopreservation	53.4 ± 1.5% motility post-thaw	Trimeche et al., 1995
		Sperm	Artificial vagina	Cryopreservation	Up to 53.4 ± 1.5% motility post-thaw	Rota et al., 2004
	<i>Equus caballus</i>	Sperm	Artificial vagina	Cryopreservation	Up to 49.3% motility post-thaw	Vieira et al., 2013
		Oocytes	Recovery of oocytes by follicle aspiration and slicing of ovaries post-mortem	Vitrification	Up to 16.7% of oocytes matured to metaphase II following in vitro maturation post-thaw	Hochi et al., 1996
	<i>Equus ferus przewalski</i>	Sperm	Electroejaculation	Cryopreservation	Up to 52.1 ± 5.4% motility	Pukazhenti et al., 2014
	<i>Equus hemionus onager</i>	Sperm	Electroejaculation	Cryopreservation	53.5 ± 6% progressive motility Birth of one foal following artificial insemination using frozen-thawed sperm	Schook et al., 2013
	<i>Equus quagga burchelli</i>	Sperm	Artificial vagina	Cryopreservation	20% motility post-thaw	Crump and Crump, 1994
	<i>Equus grevyi</i>	Sperm	Manual stimulation	Cryopreservation	Up to 70% motility post-thaw	Crump and Crump, 1994
	<i>Rhinoceros unicornis</i>	Sperm	Electroejaculation	Cryopreservation	Up to ~70% motility post-thaw	Stoops et al., 2010
		Sperm	Post-coital recovery of sperm from female	Cryopreservation	Up to 29.9 ± 2.5% motility post-thaw	O'Brien and Roth, 2000b
Rhinocerotidae	<i>Dicerorhinus sumatrensis</i>	Sperm	Electroejaculation	Cryopreservation	40% motility post-thaw	Portas et al., 2009
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	55.6 ± 1.6% motility post-thaw	O'Brien and Roth, 2000b
	<i>Ceratotherium simum cottoni</i>	Sperm	Electroejaculation	Cryopreservation	32.8% motility post-thaw	Hermes et al., 2005
	<i>Ceratotherium simum simum</i>	Sperm	Electroejaculation	Cryopreservation	49% motility post-thaw	Hermes et al., 2005
		Sperm	Electroejaculation	Cryopreservation	78% motility post-thaw. Birth of one calf following artificial insemination using frozen-thawed sperm	Hermes et al., 2008
		Sperm	Electroejaculation	Cryopreservation	~40% motility post-thaw	Pukazhenti et al., 2010
	<i>Tapirus bairdii</i>	Sperm	Electroejaculation	Cryopreservation	~40% motility post-thaw	Pukazhenti et al., 2010
<b>Order: Primates</b>						
Callitrichidae	<i>Callithrix geoffroyi</i>	Sperm	Recovery of testicular sperm post-mortem	Cryopreservation	~40% intact acrosomes post-thaw	Pothana et al., 2016

	<i>Callithrix jacchus</i>	Embryos	Laparotomy and retrograde flushing	Cryopreservation	Embryo transfer resulted in 10 pregnancies of which 8 gave birth	Hearn and Summers, 1986
		Ovarian tissue	N/A	Cryopreservation	Xenografting of frozen-thawed ovarian tissue into mice was successfully accomplished	Candy et al., 1995
Cebidae	<i>Sapajus apella</i>	Sperm	Electroejaculation	Cryopreservation	34.0 ± 10.2% motility post-thaw, which successfully fertilized oocytes with IVF	Leão et al., 2015
	<i>Saimiri sciureus collinsi</i>	Sperm	Electroejaculation	Cryopreservation	0.6 ± 1.3% motility post-thaw	Oliveira et al., 2015
		Sperm	Electroejaculation	Cryopreservation	No motility post-thaw	Oliveira et al., 2016
	<i>Saimiri vanzolinii</i>	Sperm	Electroejaculation	Cryopreservation	Up to 30% plasma integrity post-thaw	Oliveira et al., 2016
	<i>Saimiri sciureus macrodon</i>	Sperm	Electroejaculation	Cryopreservation	Up to 10% plasma integrity post-thaw	Oliveira et al., 2016
	<i>Saimiri sciureus cassiquiarensis</i>	Sperm	Electroejaculation	Cryopreservation	Up to 6% motility post-thaw	Oliveira et al., 2016
	<i>Saimiri sciureus</i>	Oocytes	Recovery of oocytes by laparotomy and aspiration	Cryopreservation	Up to 37.5% viability post-thaw. Following IVF of frozen-thawed ova, 50% became fertilized and one ovum cleaved	DeMayo et al., 1985
Cercopithecidae	<i>Macaca fascicularis</i>	Sperm	Electroejaculation	Cryopreservation	Up to 70% motility post-thaw. Fertilization of 57.1% oocytes following IVF using frozen-thawed sperm	Sankai et al., 1994
		Sperm	Electroejaculation	Cryopreservation	42.95 ± 0.6% motility post-thaw	Li et al., 2005
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	61.4% oocytes became fertilized following intra cytoplasmic sperm injection resulting in two births after embryo transfer	Ng et al., 2002
		Embryos	Recovery of oocytes via laparotomy	Cryopreservation	Up to 100% viability post-thaw	Curnow et al., 2002
		Embryos	Follicular aspiration	Cryopreservation	Up to 70% viability post-thaw. Following embryo transfer, two live births were reported	Balmaceda et al., 1986
		Ovarian tissue	Recovered by bilateral oophorectomy	Cryopreservation	Following autologous ovarian grafting, 50% of recipients showed consecutive menstrual cycles, development of follicles, and one metaphase II oocyte	Schnorr et al., 2002
	<i>Macaca mulatta</i>	Sperm	Electroejaculation	Directional freezing	63.7 ± 2.8% motility post-thaw	Si et al., 2010
		Sperm	Electroejaculation	Cryopreservation	56 ± 5% motility post-thaw. Fertilization of 82 ± 13% oocytes following IVF using frozen-thawed sperm	Si et al., 2000
		Sperm	Electroejaculation	Freeze-drying	No motility after rehydration. Fertilization of 73.1% oocytes following intra cytoplasmic sperm injection	Sánchez-Partida et al., 2008
		Embryos	Follicular aspiration	Cryopreservation	Up to 86% viability post-thaw. Live births were reported following embryo transfer	Lanzendorf et al., 1990
		Embryos	Collection of oocytes via laparoscopy	Vitrification	Up to 85% viability post-thaw. Following ET, live births were reported	Yeoman et al., 2001
		Testicular tissue	N/A	Vitrification	Following xenografting of testicular tissue, testosterone production was present	Poels et al., 2012
		Sperm	Electroejaculation	Cryopreservation	Up to 25% motility post-thaw	Roussel and Austin, 1967



	<i>Macaca silenus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Acceptable post-thaw motility. During IVF, post-thaw sperm demonstrated in vitro penetration of hamster oocytes	Durrant, 1987
	<i>Mandrillus sphinx</i>	Sperm	Recovery of sperm from testicular tissue post-mortem	Cryopreservation	~40% acrosome integrity post-thaw	Pothana et al., 2016
	<i>Papio hamadryas</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	42.5 ± 7.0% motility post-thaw	O'Brien et al., 2003
		Sperm	Spontaneous ejaculation	Cryopreservation	Up to 48.3% motility post-thaw	Kraemer and Cruz, 1969
		Embryos	Non-surgical recovery of embryos	Cryopreservation	Two live births were reported following embryo transfer	Pope et al., 1984; 1986
	<i>Erythrocebus patas</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Acceptable post-thaw motility. During IVF, post-thaw sperm demonstrated in vitro penetration of hamster oocytes	Durrant, 1987
		Sperm	Electroejaculation	Cryopreservation	Up to 23% motility post-thaw	Roussel and Austin, 1967
	<i>Chlorocebus aethiops</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 32% motility post-thaw	Sparman et al., 2007
		Embryos	N/A	Cryopreservation	Up to 42.86% viability post-thaw. No pregnancies were established following embryo transfer	Sparman et al., 2007
	<i>Macaca nemestrina</i>	Embryos	N/A	Cryopreservation	One live birth following embryo transfer was reported	Cranfield et al., 1992
Hominidae	<i>Pan troglodytes</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	82.5 ± 2.5% motility post-thaw	O'Brien et al., 2003
		Sperm	Artificial vagina	Cryopreservation	Up to 60% motility post-thaw	Younis et al., 1998
	<i>Pan paniscus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Acceptable post-thaw motility. Frozen-thawed sperm demonstrated in vitro penetration of hamster oocytes	Durrant, 1987
	<i>Gorilla gorilla</i>	Sperm	Manual stimulation	Cryopreservation	5.7 ± 6.0% motility post-thaw	O'Brien et al., 2005
		Sperm	Electroejaculation	Cryopreservation	Up to 17% motility post-thaw. During IVF, sperm cells were able to penetrate 42% zona-free hamster oocytes in vitro	Lambert et al., 1991
		Sperm	Manual stimulation	Cryopreservation	Up to 40% motility post-thaw. Following IVF, one live birth was reported	Pope et al., 1997
	<i>Hylobates lar</i>	Sperm	Manual stimulation	Cryopreservation	Up to 30% progressive motility post-thaw	Takasu et al., 2016
Lemuridae	<i>Lemur catta</i>	Sperm	Recovery of sperm post-mortem	Cryopreservation	Satisfactory results	Maksudov et al., 2008
<b>Order: Proboscidea</b>						
Elephantidae	<i>Elephas maximus</i>	Sperm	Rectal manual stimulation	Cryopreservation	58.5 ± 6.0% motility post-thaw	Saragusty et al., 2009
	<i>Loxodonta africana</i>	Sperm	Electroejaculation	Cryopreservation	~90% motility post-thaw	Gilmore et al., 1998
		Sperm	Electroejaculation	Cryopreservation	Up to 61% motility post-thaw	Hildebrandt et al., 2012
		Sperm	Electroejaculation	Cryopreservation	One pregnancy was established following artificial insemination using frozen-thawed sperm	In Hermes et al., 2013
		Sperm	N/A	Cryopreservation	Live birth following artificial insemination using frozen-thawed sperm	Saragusty et al., 2015

		Ovarian tissue	Recovery of ovarian tissue post-mortem	Cryopreservation	Xenografted into mice, where 88.7% demonstrated prolonged oestrogenic activity and the development of follicles, cumulus-like cells and one oocyte, although the oocyte was of poor quality	Gunasena et al., 1998
<b>Order: Rodentia</b>						
Caviidae	<i>Cavia porcellus</i>	Embryos	Recovery of embryos from uteri and oviducts post-mortem	Cryopreservation	64.8% intact embryos post-thaw. PROH-diluent was superior to DMSO-diluent cryoprotectant	Dorsch et al., 2008
Chinchillidae	<i>Chinchilla lanigera</i>	Sperm	Electroejaculation	Cryopreservation	~20% motility post-thaw	Ponzio et al., 2008
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to ~58% motility post-thaw	Ponce et al., 1998b
		Sperm	Electroejaculation	Cryopreservation	44.6 ± 2.2% motility post-thaw	Ponce et al., 1998a
Cricetidae	<i>Mesocricetus auratus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation and freeze-drying	Freeze-dried and frozen-thawed sperm were injected into fresh oocytes using intra cytoplasmic sperm injection, where 16.2 and 16.3% developed into blastocysts, respectively	Muneto and Horiuchi, 2011
	<i>Phodopus Campbelli</i>	Embryos	Recovery of embryos from uteri and oviducts post-mortem	Cryopreservation	Up to 100% blastocyst development post-thaw	Amstislavsky et al., 2015
	<i>Phodopus Sungorus</i>	Embryos	Recovery of embryos from uteri and oviducts post-mortem	Cryopreservation	50% blastocyst development post-thaw. Three live offspring were born following embryo transfer	Brusentsev et al., 2015
Dasyproctidae	<i>Dasyprocta sp.</i>	Sperm	Recovery of sperm from cauda epididymis post-mortem	Cryopreservation	26.5 ± 2.6% motility post-thaw	Silva et al., 2011
	<i>Dasyprocta sp.</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	26.6 ± 2.6% motility post-thaw	Silva et al., 2012
	<i>Dasyprocta leporina</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to 47.5 ± 8.4% motility post-thaw	Castelo et al., 2015
		Sperm	Electroejaculation	Cryopreservation	12.2 ± 1.3% progressive motility post-thaw	Mollineau et al., 2011
Muridae	<i>Mus musculus</i>	Sperm	Recovery of sperm from epididymis post-mortem	Freeze-drying or cryopreservation without cryoprotection	37% and 52% normal fetuses, respectively	Ward et al., 2003
		Sperm	Recovery of sperm from epididymis post-mortem	Freeze-drying	82% embryo development and up to 33% live offspring following intra cytoplasmic sperm injection and embryo transfer	Kaneko and Serikawa, 2012a
		Sperm	Recovery of sperm from epididymis post-mortem	Freeze-drying	13 and 59% of fertilized oocytes reached blastocyst stage following intra cytoplasmic sperm injection using freeze-dried sperm stored for 6 months at 4°C and -80°C, respectively	Kawase et al., 2005
		Testicular tissues	Recovery of testicular tissue post-mortem	Cryopreservation or vitrification	Eight live offspring were born following intra cytoplasmic sperm injection or round spermatid injections using frozen-thawed sperm, followed by embryo transfer	Yokonishi et al., 2014

		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	92.3% oocytes developed to two-cell stage following IVF. Up to 43.3% of embryos resulted in fertile offspring following. Sperm had been cryopreserved for >10 years	Kaneko et al., 2006
		Epididymis and testis	Recovery of sperm from epididymis and testis post-mortem	Cryopreservation	Up to 33.3% live fetuses following intra cytoplasmic sperm injection and embryo transfer. Up to 65% live fetuses after intra cytoplasmic sperm injection and embryo transfer following shipping from England to Japan	Ogonuki et al., 2006
		Whole body	Recovery of sperm post-mortem	Cryopreservation	Up to 21% normal fetuses after intra cytoplasmic sperm injection and embryo transfer following freezing at -20°C for 15 years	Ogonuki et al., 2006
		Embryos	Recovery of embryos from uteri and oviducts post-mortem	Vitrification	Up to 99.5% blastocysts after culture and $43.9 \pm 9.4\%$ birth rate after embryo transfer	An et al., 2015
		Embryos	Recovery of embryos from uteri post-mortem	Slow-freezing or vitrification	52.5% and 48.3% blastocyst hatching rate respectively	Liu et al., 2009
<i>Rattus norvegicus</i>		Sperm	Recovery of sperm from cauda epididymis post-mortem	Freeze-drying	Development of 11% offspring using intra cytoplasmic sperm injection and embryo transfer	Kaneko and Serikawa, 2012b
		Embryos	Recovery of embryos from uteri post-mortem	Double vitrification	Embryo development rates of $82.6 \pm 4.1$ , $34.4 \pm 4.8$ and $76.3 \pm 4.9\%$ were achieved for early blastocyst, blastocyst and expanding blastocyst stages respectively	Isachenko et al., 2003
		Ovaries	Post-mortem	Slow-freezing or vitrification	Up to ~29% follicle viability	Milenkovic et al., 2012
<b>Order: Scandentia</b>						
Tupaiaidae	<i>Tupaia belangeri</i>	Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to $43.3 \pm 1.8\%$ motility post-thaw. Following artificial insemination using frozen-thawed epididymal sperm, four animals exhibited fertilized oocytes with 57% fertilization rate	Ping et al., 2011
		Sperm	Recovery of sperm from epididymis post-mortem	Cryopreservation	Up to $44.9 \pm 1.8\%$ motility post-thaw. 55% fertilization rate of oocytes Following artificial insemination using frozen-thawed sperm	Ping et al., 2012

which provides incentives for scientists to continuously submit their work.

## CONCLUSION

The accelerating decline in biodiversity calls for the implementation of cryobanks and cryopreservation in conservation strategies, which have the potential to assist and improve ex situ and in situ conservation. Cryopreservation of germplasm from wild populations has been successfully implemented in ex situ breeding programs. In the class Mammalia, at least 2.7% of species has been subject to examination in which the extent of successful cryopreserva-

tion and ARTs vary. The species examined belong to less than half of all orders, and a strongly disproportionate distribution of studies across orders has been observed. The application of cryopreservation should be considered in the species-rich or non-examined orders. The cryopreservation of germplasm has in several cases been successful and resulted in successful applications of ARTs. Domesticated species and species relevant for general research have been extensively examined. Protocols for threatened species have successfully been extrapolated from these examinations, which gives incentives for future conservation of genetic diversity in threatened species. Interspecific and

intraspecific differences complicate the extrapolation of protocols from non-threatened to threatened species. One approach to be considered as a supplement to the extrapolation of protocols in closely related species is the examination and comparison of cryobiological traits. For the implementation of new genes from wild populations in ex situ breeding programs, the contamination and disease transmission risks are to be taken seriously, before routine transportation of cryopreserved material can be utilized. For the future development of cryopreservation, the alternative techniques mentioned should be considered. The development of a peer-reviewed online database should be considered, as it would offer an easy and accessible overview.

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### COMPETING INTERESTS

The authors have no competing interests to declare.

### AUTHOR CONTRIBUTIONS

SJC and MBN wrote the paper with major contributions by CRP, LT, MPK and TBS and significant contributions by CP and JS. All authors contributed to analysis, interpretations and conclusions.

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