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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°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°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°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°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 |
|-------------------------|-------------------------------|----------|-------------------------|--|---|---------------------------------|
| Order: Carnivora | | | | | | |
| 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 |

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| | <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 |

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| | <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 |

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| | | 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 |
| Order: Chiroptera | | | | | | |
| 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 |
| Order: Dasyuromorphia | | | | | | |
| 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 |
| Order: Diprotodontia | | | | | | |
| 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 |

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| | | Sperm | Recovery of sperm from epididymis post-mortem | Cryopreservation | ~45% viability post-thaw | Bickell et al., 2001 |
| Order: Lagomorpha | | | | | | |
| 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 |
| Order: Monotremata | | | | | | |
| 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 |
| Order: Peramelemorphia | | | | | | |
| Peramelidae | <i>Isodon macrourus</i> | Sperm | Recovery of sperm from epididymis post-mortem | Cryopreservation | 45% motility post-thaw | Taggart et al., 1996 |
| Order: Perissodactyla | | | | | | |
| 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 |
| Tapiridae | <i>Tapirus bairdii</i> | Sperm | Electroejaculation | Cryopreservation | ~40% motility post-thaw | Pukazhenti et al., 2010 |
| Order: Primates | | | | | | |
| Callitrichidae | <i>Callithrix geoffroyi</i> | Sperm | Recovery of testicular sperm post-mortem | Cryopreservation | ~40% intact acrosomes post-thaw | Pothana et al., 2016 |

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|-----------------|---|-------------------|--|----------------------|---|------------------------------|
| | <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 |

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| | <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 |
| Order: Proboscidea | | | | | | |
| 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 |

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|------------------------|-----------------------------|--------------------|---|--|---|----------------------------|
| | | 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 |
| Order: Rodentia | | | | | | |
| 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 |

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|--------------------------|-------------------------|-----------------------|--|--------------------------------|---|----------------------------|
| | | 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 ± 4.1 , 34.4 ± 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 |
| Order: Scandentia | | | | | | |
| 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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