



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2026; 11(1): 403-411

© 2026 VET

www.veterinarypaper.com

Received: 15-11-2025

Accepted: 20-12-2025

Lakshmi Yadav

PhD Scholar, Division of Animal
Reproduction, ICAR-Indian
Veterinary Research Institute,
Izatnagar, Bareilly, Uttar
Pradesh, India

Neeraj Srivastava

Principal Scientist, Division of
Animal Reproduction, ICAR-
Indian Veterinary Research
Institute, Izatnagar, Bareilly,
Uttar Pradesh, India

Dhaval Jitendra Kamothi

Assistant Professor, Department
of Veterinary Pharmacology and
Toxicology, College of Veterinary
Science and Animal Husbandry,
Kamdhenu University, Bhuj,
Gujarat, India

Corresponding Author:

Lakshmi Yadav

PhD Scholar, Division of Animal
Reproduction, ICAR-Indian
Veterinary Research Institute,
Izatnagar, Bareilly, Uttar
Pradesh, India

Reproductive biotechnologies for conservation of endangered mammalian species

Lakshmi Yadav, Neeraj Srivastava and Dhaval Jitendra Kamothi

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i1f.2997>

Abstract

Rapid losses of mammalian biodiversity caused by habitat conversion, illegal exploitation, and climate instability pose serious risks to ecosystem function and resilience. In this context, reproductive biotechnologies (RBTs) encompassing Assisted Reproductive Technologies (ARTs) and advanced ARTs (aARTs) have become valuable components of modern conservation practice. Established approaches such as artificial insemination, *in vitro* fertilization, embryo transfer, gamete cryopreservation, and somatic cell nuclear transfer are increasingly complemented by emerging strategies including induced pluripotent stem cells, transgenesis, and microfluidic systems. Together, these tools facilitate genetic rescue, enhance reproductive output, and enable propagation of taxa with limited or failing natural breeding. Long-term safeguarding of biodiversity is further strengthened by genome resource banking initiatives, exemplified in India by the National Wildlife Genetic Resource Bank at CCMB-LaCONES. Documented successes in species such as the black-footed ferret, Przewalski's horse, the northern white rhinoceros, and several wild felids illustrate the conservation potential of these technologies. Although biological complexity and ethical considerations remain, coordinated integration of RBTs with *in situ* and *ex situ* programs offers a viable pathway for sustaining endangered mammalian populations.

Keywords: Assisted reproductive technologies, wildlife conservation, genome resource banking, cryopreservation, advanced reproductive biotechnology

Introduction

Endangered species are defined as organisms facing an exceptionally high probability of extinction in the near future according to global conservation assessments. Present-day extinction rates greatly exceed speciation, largely as a consequence of anthropogenic pressures including habitat degradation, excessive hunting and fishing, and poaching. India, recognized as a mega-diverse nation, harbors approximately 8.6% of the world's mammalian species, 13.7% of birds, 12.2% of fishes, and 6.0% of flowering plants in 2025. At a global scale, extinction risk threatens nearly 12% of birds, 27% of mammals, and 41% of amphibians (IUCN, 2025). India alone supports around 813 globally threatened animal species, representing about 1.9% of the world's total threatened fauna (IUCN Red List Year, 2025).

The accelerating erosion of biodiversity is not only an ecological crisis but also a socioeconomic one, influencing food security, public health, climate regulation, and social equity (WHO, 2015). In addition to species-specific diseases (Shome *et al.* 1999; Rai *et al.*, 2010; Srivastava *et al.*, 2017) ^[94, 84, 99] that contribute to population decline, the pace of environmental change driven by human activity often outstrips the capacity of organisms to adapt, precipitating extinction events (Ceballos and Ehrlich, 2018) ^[17]. These realities underscore the urgent need for comprehensive conservation initiatives.

Effective animal conservation aims to maintain populations of sufficient size and genetic variability to ensure long-term viability (Comizzoli *et al.*, 2019) ^[22]. This objective requires complementary use of *in situ* strategies, which preserve species within their native habitats, and *ex situ* approaches implemented through managed programs in captivity such as zoos, Ramsars, and ecological parks. While *in situ* conservation maintains natural adaptations (Mestanza-Ramón *et al.*, 2020) ^[62], it may be insufficient for very small or fragmented

populations where genetic diversity is rapidly eroded (Felix *et al.*, 2007) [28]. Captive breeding, though successful in certain cases, can suffer from restricted gene pools, resulting in inbreeding depression and reduced fertility (Saragusty *et al.*, 2020) [92]. Increased homozygosity further compromises adaptive capacity and reproductive performance (Wildt *et al.*, 2010) [113].

For species in which conventional breeding programs have failed (Lees and Wilcken, 2009) [53], the preservation of genetic material through cryopreservation and cryobanking storage of viable cells and tissues at -196°C in liquid nitrogen or in ultra-low freezers combined with ARTs and aARTs provides an alternative means of safeguarding genotypes that would otherwise be lost (Mitchell & Williams, 2022) [63]. ARTs encompass techniques that utilize oocytes and spermatozoa to generate offspring, including artificial insemination (AI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), embryo transfer (ET), and cloning.

Assisted Reproductive Technologies for Endangered Species Conservation

ARTs markedly enhance reproductive efficiency and represent powerful tools for conserving both domestic and wild animal populations at risk of extinction (Bolton *et al.*, 2022) [10]. These technologies help overcome limitations such as poor semen quality, low sperm output, infertility, small population size, and loss of genetic diversity in threatened species (Swanson, 2023) [101].

ART development is often described in successive generations. Early applications focused on gamete and embryo cryopreservation and AI, while later advancements incorporated ultrasonography and multiple ovulation and embryo transfer (MOET). Subsequent progress enabled ovum pick-up (OPU) and IVF, culminating in advanced ARTs (aARTs) that include cloning by nuclear transfer, transgenesis, stem cell technologies, and marker-assisted selection (MAS) (Bertolini and Bertolini, 2009) [7]. A wide array of reproductive interventions like estrus synchronization, superovulation, non-surgical embryo recovery and transfer, oocyte retrieval, *in vitro* maturation, cloning, and nanotechnology are now applied toward conservation goals (Bisla *et al.*, 2021; Poonia *et al.*, 2023) [8, 76]. Continuous refinement of *in vitro* embryo production and cryopreservation protocols has further improved outcomes (Mohammed *et al.*, 2005; Arav, 2014) [64, 3]. Techniques such as IVF, subzonal sperm injection, and ICSI have expanded fertilization options (Gordon, 2003) [32]. Nevertheless, the effectiveness of both ARTs and aARTs is constrained by limited knowledge of reproductive physiology in many species; detailed understanding exists for only about 250 species (Herrick, 2019) [36].

Artificial Insemination

Artificial insemination was the earliest widely adopted reproductive technology, involving semen collection from males followed by deposition into females during the receptive phase of the reproductive cycle (Manafi, 2011) [59]. Owing to its relative simplicity and minimal invasiveness, AI is the most broadly applicable ART for wildlife (Comizzoli, 2015) [21]. A major conservation benefit of AI lies in mitigating genetic deterioration caused by population

fragmentation. In small or isolated populations, temporary capture of females for insemination with semen from genetically valuable males maintained in captivity can facilitate gene flow, after which females may return to their natural habitats to give birth (Pukazhenthil and Wild, 2004) [82].

AI has resulted in live births across a wide spectrum of wildlife species, including Blackbuck (Holt *et al.*, 1988) [67], African lion (Bowen *et al.*, 1982) [14], Black-footed ferret (Howard *et al.*, 1991) [43], Ocelot and Eld's deer (Monfort *et al.*, 1993) [66], Cheetah (Wildt *et al.*, 1997) [114], Marmoset (Morrell *et al.*, 1998) [67], Poitou donkey (Trimeche *et al.*, 1998) [109], Asian and African elephants (Olson and Wiese, 2000; Brown *et al.*, 2009) [74, 39], Oryx (Morrow *et al.*, 2000) [68], and Giant panda (Masui *et al.*, 1989) [61]. Despite more than 50 reported species successes, routine AI application remains largely confined to commercially managed wildlife such as Red deer, Fallow deer, White-tailed deer, and Reindeer. Further investigation is required to address constraints, including the effects of anesthesia used during electro-ejaculation on semen quality (Hildebrandt *et al.*, 2000) [37].

Embryo Transfer and *In vitro* Fertilization

Embryo transfer enables the production of multiple offspring from genetically superior females (Gordon, 2003; Mohammed *et al.*, 2011) [32, 65]. Embryos are recovered from the oviduct or uterus using surgical or non-surgical techniques and can be transferred to recipient females or cryopreserved for future use (Mohammed *et al.*, 2011) [65].

IVF involves fertilizing oocytes with fresh or cryopreserved spermatozoa in a controlled culture environment (Mohammed *et al.*, 2005) [64]. Prior to fertilization, spermatozoa undergo *in vitro* capacitation commonly achieved via swim-up or Percoll gradient methods to acquire the ability to penetrate the zona pellucida (Gordon, 2003; Mohammed *et al.*, 2005; Jerome *et al.*, 2012) [32, 64, 47]. Capacitation induces physiological changes such as enhanced motility, calcium influx, and protein interactions (Donà *et al.*, 2020) [27]. Following co-incubation of gametes for approximately 8-22 hours, fertilized oocytes (zygotes) are formed (Greda *et al.*, 2006) [33]. Mature oocytes used for IVF may be collected from slaughterhouse ovaries and matured *in vitro* or retrieved from live females via OPU (Mohammed *et al.*, 2005; Aller *et al.*, 2010) [65, 1]. IVF is particularly valuable for producing embryos from terminal or compromised females and for conservation breeding.

While AI primarily facilitates dissemination of male genetics, embryo-based techniques like IVF, ET, and cryopreservation enable representation of female genetic contributions. These methods are, however, more invasive, technically demanding, and costly. Interspecies embryo transfer has therefore been explored as a conservation strategy, wherein embryos from endangered species are transferred to surrogate females of related species. Landmark successes include a gaur calf born to a Holstein cow (Stover *et al.*, 1981) [100], Armenian red sheep gestated by domestic sheep (Coonrod *et al.*, 1994) [25], and Spanish ibex carried by domestic goats (Fernandez-Arias *et al.*, 1996) [29]. Subsequent achievements have involved banteng, zebra, Indian desert cat, and European mink embryos carried by surrogate species (Loskutoff, 2003; Amstislavsky *et al.*, 2004) [58, 2].

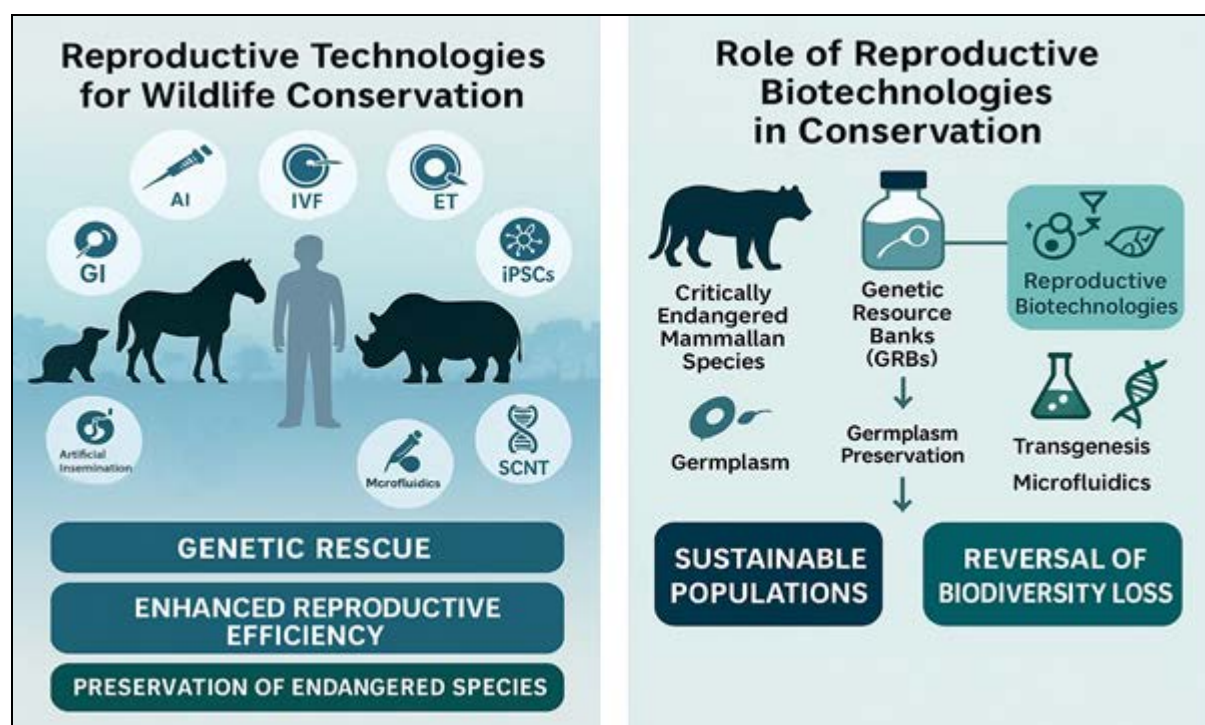


Fig 1: Overview of reproductive biotechnologies applied in the conservation of endangered mammalian species.

Another application of IVF is *in vitro* maturation (IVM) of oocytes recovered from females that die unexpectedly, a strategy termed “genetic recovery” (Galli *et al.*, 2003; Pukazhenthi and Wildt, 2004) [30, 82]. This approach has yielded live offspring in species such as gaur (Johnston *et al.*, 1994) [50], Armenian red sheep, llama, and European mouflon (Ptak *et al.*, 2002) [81]. Success depends on donor age, health, and culture conditions (Christensen *et al.*, 1993) [20]. IVF and *in vitro* production have also been applied in Armenian red sheep, Sika deer, European mouflon, African elephant, minke whale, gorilla, and zebra (Comizzoli *et al.*, 2001; Ptak *et al.*, 2002; Tetsuka *et al.*, 2004) [23, 81, 104].

Genome Resource Banking (GRB) or Biobanking

Given the rapid pace of extinction, it is impractical to design tailored conservation programs for all threatened species. Genome resource banking offers a strategic means of preserving biodiversity by storing genetic materials like DNA, RNA, semen, oocytes, embryos, tissues, and cells at ultra-low temperatures near -196°C (Wisely *et al.*, 2015) [116]. GRBs function as interfaces between *in situ* and *ex situ* efforts, facilitating exchange of genetic diversity and creation of global gene pools (Hanks, 2001; Holt and Pickard, 2004) [35, 41]. Worldwide, 22 institutions currently maintain biological repositories for wildlife ART applications. The Frozen Zoo at the San Diego Zoo Institute for Conservation Research houses more than 10,000 living cell cultures and gametes from nearly 1,000 taxa. In India, CCMB-LaCONES hosts the National Wildlife Genetic Resource Bank, the country’s sole dedicated wildlife biobanking facility.

Semen Cryopreservation

Semen cryopreservation preserves spermatozoa at sub-zero temperatures, commonly -196°C in liquid nitrogen, enabling long-term storage (Murray and Gibson, 2022) [71]. Cryoprotective agents are essential to maintain sperm viability during freezing and thawing (Tamburrino *et al.*, 2023) [103]. This approach underpins numerous conservation

and breeding applications, including AI and IVF (Prieto *et al.*, 2014) [80].

Cryopreserved spermatozoa form the basis of living cryobanks that support genetic management across taxa (Browne *et al.*, 2019; Asturiano *et al.*, 2017; Xin *et al.*, 2017; Rickard *et al.*, 2022; Cardoso *et al.*, 2020) [15, 17, 104, 86, 16]. Because sperm are haploid and continuously produced, they allow broad genetic representation. Advances in cryopreservation protocols (Pande *et al.*, 2015; Katiyar *et al.*, 2022) [75, 51] support the use of well-studied domestic species as models for related wildlife, including equine protocols for rhinoceros (Reid *et al.*, 2009) [85], bovine models for gazelles (Saragusty *et al.*, 2006) [93], and domestic ferrets for Black-footed ferrets (Howard *et al.*, 2016) [42]. Comparable adaptations have been applied to Asian elephants, giant pandas, bees, killer whales, and bottlenose dolphins (Saragusty *et al.*, 2009; Martin-Wintle *et al.*, 2019; Comizzoli *et al.*, 2019; Robeck *et al.*, 2011) [94, 60, 22, 87].

Oocyte Cryopreservation

Oocytes represent another valuable genetic resource for conservation. However, their large size and low surface area-to-volume ratio make them particularly susceptible to intracellular ice formation during freezing (Borini and Bianchi, 2012) [13]. Variable membrane permeability to cryoprotectants further complicates preservation, often resulting in reduced fertilization rates after thawing (Arav, 2014; Tharasanit and Thuwanut, 2021) [3, 105]. Despite these challenges, progress has been achieved in multiple species (Felix *et al.*, 2007) [28]. In felids, oocyte cryopreservation remains largely experimental, with post-thaw developmental competence still limited (Jewgenow and Zahmel, 2020) [48]. Domestic cats are therefore used as models to refine protocols applicable to wild felids (Fernandez-Gonzalez *et al.*, 1996) [29].

Gonadal Tissue Cryopreservation

Preservation of gonadal tissue provides an alternative to storing mature gametes, enabling retention of the full spectrum of germ cell stages (Comizzoli *et al.*, 2012) [24].

Cryopreservation of Testicular Tissue

Testicular tissue cryopreservation enables recovery of genetic material from valuable males that die unexpectedly. In domestic cats, viable spermatozoa retrieved from cryopreserved testicular tissue have been used via ICSI to produce live offspring (Tharasanit *et al.*, 2012)^[106]. Testicular sperm often exhibit higher post-thaw viability (Chatdarong, 2011)^[18]. Techniques such as *in vitro* culture of thawed tissue and ultra-rapid freezing show promise (Sato *et al.*, 2011; Pothana *et al.*, 2017; da Silva *et al.*, 2020)^[95, 78, 11]. Reports span numerous wildlife species, including jungle cat, lion, leopard, Rusa deer, Fea's muntjac, Sumatran serow, and Eld's deer (Thuwanut, 2013)^[107]. Comparable success has been observed in nonhuman primates using slow-freezing protocols (Pothana *et al.*, 2016)^[94].

Cryopreservation of Ovarian Tissue

Ovarian tissue cryopreservation preserves immature follicles that would otherwise never contribute to reproduction, offering a means to safeguard fertility from neonatal, prepubertal, or non-breeding females (Santos *et al.*, 2010)^[91]. This technique has been applied across species such as White-tailed deer, domestic cat, collared peccary, yellow-toothed cavy, donkey, and cattle (Vasconcelos *et al.*, 2013; Praxedes *et al.*, 2017; Lopes *et al.*, 2018; Lima *et al.*, 2019; Gastal *et al.*, 2018; Mouttham & Comizzoli, 2016)^[110, 79, 57, 54, 31, 69]. Following death or neutering, cortical tissue containing follicles is dissected, sectioned, and cryopreserved (Hinkle *et al.*, 2021)^[38]. Projects such as the Rhino Fertility Project are advancing *in vitro* culture systems for critically endangered species like the northern white rhinoceros, although challenges related to survival and developmental competence remain.

Advanced Assisted Reproductive Technology

Reproductive Cloning

Reproductive cloning via somatic cell nuclear transfer (SCNT) involves introducing a somatic cell nucleus into an enucleated oocyte to generate an embryo genetically identical at the nuclear level to the donor. The reversibility of cellular differentiation was first demonstrated in *Xenopus laevis* (Gurdon *et al.*, 1975)^[34], a discovery later recognized with the Nobel Prize in 2012. Mammalian cloning was achieved with the sheep (Wilmut *et al.*, 1997)^[115]. SCNT can be performed by microinjection or electrofusion, with species-specific optimization of oocyte maturation and activation protocols (Wakayama *et al.*, 1999; Liu *et al.*, 2015; Qu *et al.*, 2020)^[11, 56, 83].

For endangered species, limited availability of oocytes

necessitates interspecific SCNT (iSCNT), yielding offspring with donor nuclear DNA but recipient mitochondrial DNA (Lagutina *et al.*, 2013)^[52]. Such individuals are regarded by IUCN as functional proxies capable of restoring ecological roles, though they are not genetically identical to extinct species (IUCN, 2016). Live progeny via iSCNT have been reported in yak, African wildcat, sand cat, bucardo, Bactrian camel, grey wolf, coyote, mouflon, river buffalo, and caracal (Lagutina *et al.*, 2013)^[52]. SCNT has supported rescue efforts for species such as the Black-footed ferret and Przewalski's horse (Sandler *et al.*, 2021; Novak *et al.*, 2023)^[90, 72]. Complications including increased pregnancy loss have been linked to immunological incompatibilities, prompting exploration of alternative strategies such as inner cell mass transfer (Rutigliano *et al.*, 2022; Saragusty *et al.*, 2020)^[88, 92].

Stem Cell Technology

Stem cell approaches offer additional avenues for preserving genetic diversity. While embryonic stem cells can generate germ cells (Toyooka *et al.*, 2003)^[108], their derivation is unsuitable for endangered species. Induced pluripotent stem cells (iPSCs), reprogrammed from adult somatic cells (Takahashi and Yamanaka, 2006)^[102], provide a viable alternative, although differences from embryonic stem cells may affect immunogenicity and differentiation (Yamanaka, 2012; Okita *et al.*, 2012)^[102, 71]. Fibroblasts from domestic and endangered species have demonstrated post-thaw viability and reprogramming potential (Li *et al.*, 2009; Na *et al.*, 2010; Liu *et al.*, 2015)^[56]. iPSCs derived from frozen fibroblasts of the Drill and the northern white rhinoceros exemplify the promise of this technology for conservation (Ben-Nun *et al.*, 2011)^[5].

Microfluidics

Microfluidic technologies developed for human and domestic animal reproduction offer new opportunities for wildlife conservation. These miniaturized systems manipulate microliter to femtoliter volumes with high precision, enabling improved assessment of reproductive status and gamete quality. Applications include microsurgical sperm extraction, gamete selection, oocyte quality assessment, IVF-on-a-chip platforms, and controlled culture environments (Berendsen *et al.*, 2019; Yanez and Camareillo, 2017; Huang *et al.*, 2018; Sharma, 2020; Samuel *et al.*, 2020)^[6, 119, 44, 89]. Microfluidics also enhances optimization of cryopreservation protocols and reduces contamination risk. Integrated within broader conservation strategies, these tools contribute to a versatile biotechnological toolkit for biodiversity preservation (Corlett, 2017)^[26].

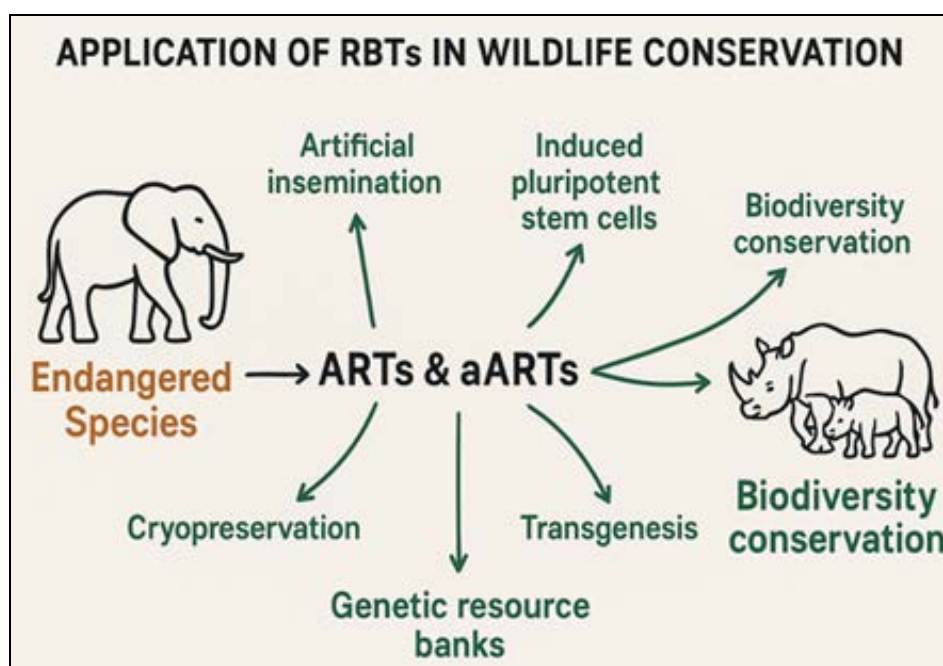


Fig 2: Advanced assisted reproductive technologies and emerging biotechnological tools for endangered mammalian species conservation.

Conclusion

ARTs and aARTs provide substantial opportunities for conserving endangered mammals, with significant progress achieved over recent decades. Incorporation of tissue cryobanking into conservation planning is increasingly important as a reservoir of genetic diversity for future use (Comizzoli & Holt, 2019) ^[21]. Effective strategies should combine in situ and ex situ approaches with reproductive biotechnologies such as AI, gamete micromanipulation, cryopreservation, *in vitro* culture, and tissue grafting. Beyond facilitating reproduction, these techniques also generate valuable insights into the reproductive biology of wild species. Although ethical considerations and potential risks remain, continued research, collaboration, and public engagement can maximize the benefits of these technologies for safeguarding global biodiversity.

Future Prospects

Integration of advanced ARTs with conventional conservation measures is expected to strengthen biodiversity protection for future generations. Further development of alternative long-term biomaterial storage methods is required where existing technologies are insufficient. A major limitation remains the paucity of reproductive physiological data for many wild species, hindering optimization of reproductive interventions. Emerging advances, particularly in SCAT combined with gene-editing technologies, hold promise for establishing genetically robust, self-sustaining populations of critically endangered mammals.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Aller JF, Callejas SS, Alberio RH, Alberio RH. Efficiency of *in vitro* embryo production using oocytes obtained by ovum pick-up from different categories of beef cows. *Animal Reproduction*. 2010;7(1):59-64.
2. Amstislavsky S, Aalto J, Jarvinen M, Lindeberg H, Valtonen M, Zudova G, Ternovskaya Y. Transfer of European mink (*Mustela lutreola*) embryos into hybrid recipients. *Theriogenology*. 2004;62:458-467.
3. Arav A. Cryopreservation of oocytes and embryos. *Theriogenology*. 2014;81:96-102.
4. Asturiano JF, Cabrita E, Horváth Á. Progress, challenges and perspectives on fish gamete cryopreservation: a mini-review. *Gen Comp Endocrinol*. 2017;245:69-76.
5. Ben-Nun IF, Mortimer D, Della-Togna G. Induced pluripotent stem cells from highly endangered species. *Nature Methods*. 2011;8:829-831.
6. Berendsen JTW. Separation of spermatozoa from erythrocytes using their tumbling mechanism in a pinch flow fractionation device. *Microsyst Nanoeng*. 2019;5:24.
7. Bertolini M, Bertolini LR. The potential of reproductive technologies for animal conservation and the need for their careful implementation. *Theriogenology*. 2009;71(1):158-165.
8. Bisla A, Rautela R, Yadav V, Saini G, Singh P, Ngou AA, Kumar A, Srivastava N. Synthesis of iron oxide nanoparticles-antiubiquitin antibodies conjugates for depletion of dead/damaged spermatozoa from buffalo (*Bubalus bubalis*) semen. *Biotechnology and Applied Biochemistry*. 2021;68(6):1453-1468.
9. Bhak J, Lee SL, Kim JS, Park JH, Kim DH, Lee YM. Development of bovine embryos following chemical activation with ionomycin and 6-DMAP. *Animal Reproduction Science*. 2006;92(1-2):45-57.
10. Bolton LA, Comizzoli P, Curry E, Holt WV. Reproductive science in wildlife conservation: progress and potential. *Conservation Science and Practice*. 2022;4(3):e12678.
11. Borges AA, Silva CM, Santos DS, Assumpção ME. Chemical activation of porcine oocytes: comparative effects of ionomycin and 6-DMAP. *Zygote*. 2020;28(1):48-56.
12. Borges AA, Pereira AF. Potential role of intraspecific and interspecific cloning in the conservation of wild mammals. *Zygote*. 2019;27:111-117.

13. Borini A, Bianchi V. Cryopreservation of oocytes and embryos. In: Gurtin ML, editor. Human Assisted Reproductive Technology: Future Trends in Laboratory and Clinical Practice. Cambridge: Cambridge University Press; 2012. p.85-95.
14. Bowen MJ, Platz CC, Kraemer DC. Successful artificial insemination and embryo collection in the African lion (*Panthera leo*). Proc Am Assoc Zoo Vet. 1982;57-59.
15. Browne RK, Silla AJ, Upton R, Della-Togna G, Marcec-Greaves R, Shishova NV. Sperm collection and storage for the sustainable management of amphibian biodiversity. Theriogenology. 2019;133:187-200.
16. Cardoso B, Sánchez-Ajofrín I, Castaño C, García-Álvarez O, Estes MC, Maroto-Morales A. Optimization of sperm cryopreservation protocol for peregrine falcon (*Falco peregrinus*). Animals. 2020;10:691.
17. Ceballos G, Ehrlich PR. The misunderstood sixth mass extinction. Science. 2018;360(6393):1080-1081.
18. Chatdarong K. Cryopreservation of feline testicular tissues and application in reproductive biotechnology. Thai Journal of Veterinary Medicine. 2011;41(1):1-7.
19. Choudhary KK, Kavya KM, Jerome A, Sharma RK. Advances in reproductive biotechnologies. Vet World. 2016;9:388-395.
20. Christensen M, Schmidt M, Hess D, Alak B. Oocyte nuclear maturation *in vitro* in the African (*Loxodonta africana*) and Asian elephant (*Elephas maximus*). Biol Reprod. 1993;48:88-95.
21. Comizzoli P. Biobanking efforts and new advances in male fertility preservation for rare and endangered species. Asian Journal of Andrology. 2015;17:640-645.
22. Comizzoli P, Brown JL, Holt WV. Reproductive science as an essential component of conservation biology: new edition. In: Comizzoli P, Brown JL, Holt WV, editors. Reproductive Sciences in Animal Conservation. Cham: Springer International Publishing; 2019.
23. Comizzoli P, Mermillod P, Cognie Y, Chai N, Legendre X, Mauge R. Successful *in vitro* production of embryos in the red deer (*Cervus elaphus*) and the sika deer (*Cervus nippon*). Theriogenology. 2001;55:649-659.
24. Comizzoli P, Songsasen N, Wildt DE. Protecting and extending fertility for females of wild and endangered mammals. Cancer Treatment and Research. 2012;156:87-100.
25. Coonrod SA, Flores-Foxworth G, Moreno JF, Westhusin M, Byrd SR, Kraemer DC. Birth of Armenian red sheep (*Ovis orientalis*) lambs to domestic sheep (*Ovis aries*) following interspecific transfer of IVM-IVF derived embryos. Theriogenology. 1994;41:182.
26. Corlett RT. A bigger toolbox: biotechnology in biodiversity conservation. Trends Biotechnol. 2017;35(1):55-65.
27. Donà G, Fiore C, Foresta C. The role of calcium in sperm capacitation and fertilization. Cell Tissue Res. 2020;380(2):325-344.
28. Felix A, Andrabi S, Maxwell W. A review on reproductive biotechnologies for conservation of endangered mammalian species. Animal Reproduction Science. 2007;99(3-4):223-243.
29. Fernandez-Arias A, Folch J, Alabart JL, Ramon J. Successful interspecific embryo transfer between Spanish ibex (*Capra pyrenaica*) and domestic goat (*Capra hircus*) using microosmotic pumps for FSH administration. Theriogenology. 1996;45:247.
30. Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S, Lagutina I, Lazzari G. Bovine embryo technologies. Theriogenology. 2003;59:599-616.
31. Gastal MO, Gastal EL, Ginther OJ. Cryopreservation of epididymal sperm from white-tailed deer (*Odocoileus virginianus*): effects of collection method and extenders. Theriogenology. 2018;106:54-61.
32. Gordon I. Establishing pregnancies with IVP embryos. In: Laboratory Production of Cattle Embryos. 2nd ed. 2003. p.303-321.
33. Greda P, Vassena R, Latham KE. Noncanonical transcription initiation during zygotic genome activation in mice. Molecular and Cellular Biology. 2006;26(24):10322-10328.
34. Gurdon JB, Laskey RA, Reeves OR. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. Journal of Embryology and Experimental Morphology. 1975;34(1):93-112.
35. Hanks J. Conservation strategies for Africa's large mammals. Reprod Fertil Dev. 2001;13:459-468.
36. Herrick JR. Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. Biology of Reproduction. 2019;100:1158-1170.
37. Hildebrandt TB, Brown JL, Hermes R, Goritz F. Ultrasound as an important tool for the development and application of reproductive technologies in non-domestic species. Theriogenology. 2000;53:157-172.
38. Hinkle K. Cryopreservation of ovarian tissue for pediatric fertility. Biopreservation and Biobanking. 2021;19:130-135.
39. Holt WV, Lloyd RE. Artificial insemination for the conservation of endangered mammals. In: Holt WV, Brown JL, Comizzoli P, editors. Reproductive sciences in animal conservation: progress and prospects. New York, NY: Springer; 2009. p.51-72.
40. Holt WV, Moore HDM, North RD, Hartman TD, Hodges JK. Hormonal and behavioural detection of oestrus in blackbuck (*Antelope cervicapra*) and successful artificial insemination with fresh and frozen semen. J Reprod Fert. 1988;82:717-725.
41. Holt WV, Pickard AR, Prather RS. Wildlife conservation and reproductive cloning. Reproduction. 2004;127:317-324.
42. Howard JG. Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. Anim Conserv. 2016;19:102-111.
43. Howard JG, Bush M, Morton C, Morton F, Wentzel K, Wildt DE. Comparative semen cryopreservation in ferrets (*Mustela putorius furo*) and pregnancies after laparoscopic intrauterine insemination with frozen-thawed spermatozoa. J Reprod Fert. 1991;92:109-118.
44. Huang HY. Dielectrophoretic microfluidic device for *in vitro* fertilization. Micromachines (Basel). 2018;9:135.
45. IUCN. IUCN SSC Guiding Principles on Creating Proxies of Extinct Species. IUCN; 2016.
46. IUCN. IUCN Red List of Threatened Species [Internet]. IUCN; 2025.
47. Jerome J, Srivastava N. Prostaglandins vis-à-vis bovine embryonic mortality: a review. Asian Pacific Journal of Reproduction. 2012;1(3):238-246.

48. Jewgenow K, Zahmel J. Preservation of female genetic resources in feline species. *Theriogenology*. 2020;156:124-129.
49. Jiménez-Rabadán P, Ramón M, García-Álvarez O, Maroto Morales A, del Olmo E, Pérez-Guzmán MD, et al. Effect of semen collection method (artificial vagina vs. electroejaculation), extender and centrifugation on post-thaw sperm quality of Blanca-Celtibérica buck ejaculates. *Anim Reprod Sci*. 2012;132:88-95.
50. Johnston LA, Parrish JJ, Monson R, Leibfried-Rutledge L, Susko-Parrish JL, Northey DL, et al. Oocyte maturation, fertilization and embryo development *in vitro* and *in vivo* in the gaur (*Bos gaurus*). *J Reprod Fert*. 1994;100:131-136.
51. Katiyar R, Ghosh SK, Karikalan M, Kumar A, Pande M, Gameda AI, Srivastava N. Evidence of Humanin-like peptide and Humanin-mediated cryosurvival of spermatozoa in buffalo bulls. *Theriogenology*. 2022;194:13-26.
52. Lagutina I, Fulka H, Lazzari G, Galli C. Interspecies somatic cell nuclear transfer: advancements and problems. *Cellular Reprogramming*. 2013;15(5):374-384.
53. Lees C, Wilcken J. Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook*. 2009;43(1):6-18.
54. Lima GL, Silva AM, Silva TF, Santos EAA, Oliveira MF. Sperm cryopreservation in collared peccaries (*Pecari tajacu*): extender comparison and fertility evaluation. *Theriogenology*. 2019;128:157-164.
55. Liu H, Li Y, Yang J, Zhao Y. Parthenogenetic activation of primate oocytes using ionomycin and 6-DMAP. *Journal of Assisted Reproduction and Genetics*. 2018;35(7):1275-1283.
56. Liu T. Factors determining the efficiency of porcine somatic cell nuclear transfer: data analysis with over 200,000 reconstructed embryos. *Cellular Reprogramming*. 2015;17:463-471.
57. Lopes FRR, Silva AM, Souza AL, Silva AR. Cryopreservation of donkey (*Equus asinus*) epididymal sperm using different extenders and freezing protocols. *Animal Reproduction Science*. 2018;189:41-48.
58. Loskutoff NM. Role of embryo technologies in genetic management and conservation of wildlife. In: Holt WV, Pickard AR, Rodger JC, Wildt DE, editors. *Reproductive Science and Integrated Conservation*. Cambridge: Cambridge University Press; 2003. p.183-194.
59. Manafi M. Artificial insemination in farm animals. *Intech Open*; 2011.
60. Martin-Wintle MS, Shepherdson DJ, Zhang G, Zhang H, Li D, Owen MA. Factors influencing reproductive success in captive giant pandas: role of husbandry and artificial insemination. *Zoo Biology*. 2019;38(1):13-29.
61. Masui M, Hiramatsu H, Nose N, Nakazato R, Sagawa Y, Tajima H, Saito K. Successful artificial insemination in the giant panda (*Ailuropoda melanoleuca*) at Ueno Zoo. *Zoo Biol*. 1989;8:17-26.
62. Mestanza-Ramón C, Henkanaththegedara SM, Váscquez Duchicela P, Vargas Tierras Y, Sánchez Capa M, Constante Mejía D, Jimenez Gutierrez M, Charco Guamán M, Mestanza Ramón P. In-situ and ex-situ biodiversity conservation in Ecuador: a review of policies, actions and challenges. *Diversity*. 2020;12(8):315.
63. Mitchell AL, Williams D. Advances in reproductive cryotechnology for wildlife conservation. *Journal of Conservation Biology*. 2022;36(2):123-135.
64. Mohammed AA, Karasiewicz J, Papis K. Oocyte maturation in the presence of randomly pooled follicular fluid increases bovine blastocyst yield *in vitro*. *J Anim Feed Sci*. 2005;14:501.
65. Mohammed AA, Karasiewicz J, Papis K. Cryopreservation and transfer of embryos in domestic animals: recent advances. *Journal of Animal and Veterinary Advances*. 2011;10(14):1742-1754.
66. Monfort SL, Asher GW, Wildt DE, Wood TC, Schiewe MC, Williamson LR, Bush M, Rall WF. Successful intrauterine insemination of the Eld's deer (*Cervus eldi*) with frozen-thawed spermatozoa. *J Reprod Fert*. 1993;99:459-465.
67. Morrell JM, Nubbemeyer R, Heistermann M, Rosenbusch J, Kuderling I, Holt W, Hodges JK. Artificial insemination in *Callithrix jacchus* using fresh or cryopreserved spermatozoa. *Anim Reprod Sci*. 1998;52:165-174.
68. Morrow CJ, Wolfe BA, Roth TL, Wildt DE, Bush M, Blumer ES, Atkinson MW, Monfort SL. Comparing ovulation synchronization protocols for artificial insemination in the scimitar-horned oryx (*Oryx dammah*). *Anim Reprod Sci*. 2000;59:71-86.
69. Mouttham L, Comizzoli P. Sperm cryopreservation and thawing procedures in domestic cats: a model for endangered felids. *Cryobiology*. 2016;73(1):111-117.
70. Mrowiec P, Bugno-Poniewierska M, Młodawska W. The perspective of the incompatibility of nucleus and mitochondria in interspecies somatic cell nuclear transfer for endangered species. *Reprod Domest Anim*. 2021;56:199-207.
71. Murray KA, Gibson MI. Chemical approaches to cryopreservation. *Nat Rev Chem*. 2022;6(8):579-593.
72. Novak BJ, et al. Somatic cell nuclear transfer in the Przewalski's horse: a breakthrough in species recovery. *Journal of Wildlife Conservation Science*. 2023;41(4):250-262.
73. Okita K, Nagata N, Yamanaka S. Immunogenicity of induced pluripotent stem cells. *Circulation Research*. 2011;109:720-721.
74. Olson D, Wiese RJ. State of the North American African elephant population and projections for the future. *Zoo Biol*. 2000;19:311-320.
75. Pande M, Srivastava N, Rajoriya JS, Ghosh SK, Prasad JK, Ramteke SS. Effects of degasified extender on quality parameters of cryopreserved bull spermatozoa. *Int J Vet Sci Res*. 2015;1(3):70-78.
76. Poonia B, Sharma RK, Yadav VP, Kumar S. Recent advances in assisted reproductive technologies for conservation of endangered animal species. *J Anim Reprod Biotechnol*. 2023;38(1):1-15.
77. Pothana L, Siva Kumar D, Reddy KS, Reddy NS. Cryopreservation of testicular tissue and its applications in conservation of endangered species: a review. *J Anim Res*. 2016;6(5):863-871.
78. Pothana L, Siva Kumar D, Reddy KS, Reddy NS. Testicular tissue cryopreservation and its application in fertility preservation: a review. *Vet World*. 2017;10(9):1105-1111.
79. Praxedes ÉCG, Silva AM, Oliveira MF, Silva AR. Cryopreservation of epididymal sperm from yellow-

- toothed caviés (*Galea musteloides*): an alternative model for wild rodents. *Cryobiology*. 2017;74:98-103.
80. Prieto MT, Sanchez-Calabuig MJ, Hildebrandt TB, Santiago-Moreno J, Saragusty J. Sperm cryopreservation in wild animals. *Eur J Wildl Res*. 2014;60(6):851-864.
 81. Ptak G, Clinton M, Barboni B, Muzzeddu M, Cappai P, Tischner M. Preservation of the wild European mouflon: the first example of genetic management using a complete program of reproductive biotechnologies. *Biol Reprod*. 2002;66(3):796-801.
 82. Pukazhenthi BS, Wildt DE. Which reproductive technologies are most relevant to studying, managing and conserving wildlife? *Reprod Fertil Dev*. 2004;16:33-46.
 83. Qu P, Crichton E, Armstrong D, Wildt DE. Melatonin protects rabbit somatic cell nuclear transfer (SCNT) embryos from electrofusion damage. *Sci Rep*. 2020;10:2186.
 84. Rai RB, Srivastava N, Jaisunder AK, Kundu A, Jeykumar S. Stephanofilariasis in bovines: prevalence, control and eradication in Andaman and Nicobar Islands, India. *Indian J Anim Sci*. 2010;80(6):500.
 85. Reid CE, Hermes R, Blottner S, Goeritz F, Wibbelt G, Walzer C, Bryant BR, Portas TJ, Streich WJ, Hildebrandt TB. Split-sample comparison of directional and liquid nitrogen vapor freezing methods on post-thaw semen quality in white rhinoceroses (*Ceratotherium simum simum* and *C. s. cottoni*). *Theriogenology*. 2009;71(2):275-291.
 86. Rickard JP, Pool K, De Graaf SP, Portas T, Rourke N, Wiesner M, Hildebrandt TB, Göritz F, Hermes R. Increasing the yield and cryosurvival of spermatozoa from rhinoceros ejaculates using the enzyme papain. *Biology*. 2022;11:154.
 87. Robeck TR, Steinman KJ, O'Brien JK, Katsumata E, Dunn JL, Schmitt TL, Osborn S. Assisted reproductive technologies (ART) in the bottlenose dolphin (*Tursiops truncatus*) and killer whale (*Orcinus orca*): current knowledge on factors affecting semen collection, freezing and artificial insemination success. *Reprod Domest Anim*. 2011;46(Suppl 6):70-78.
 88. Rutigliano HM, Takahashi MB, Yang X. Immune responses and pregnancy loss in cattle following somatic cell nuclear transfer: role of MHC class I and inflammatory cytokines. *Theriogenology*. 2022;181:45-53.
 89. Samuel R. Microfluidic system for rapid isolation of sperm from microdissection TESE specimens. *Urology*. 2020;140:70-76.
 90. Sandler JE, Howard JG, Santymire RM, Novak BJ. Genetic rescue of the black-footed ferret using somatic cell nuclear transfer. *Conserv Sci Pract*. 2021;3(12):e527.
 91. Santos RR, Brown JL, Alvarado TL. Cryopreservation of ovarian tissue: an emerging technology for female germline preservation of endangered species and breeds. *Anim Reprod Sci*. 2010;122(3-4):151-163.
 92. Saragusty J, Diecke S, Drukker M, Durrant B, Galli C, Gosden R, Holtze S, Lonergan P, Loring JF, Novak BJ, Ryder OA. Rewinding the process of mammalian extinction. *Zoo Biol*. 2020;39(1):1-15.
 93. Saragusty J, Gacitua H, Rozenboim I, Arav A, Yavin S. Towards standardization of sperm cryopreservation protocols: the bovine model as a case study for wild species. *Theriogenology*. 2006;66(6-7):1718-1726.
 94. Saragusty J, Hildebrandt TB, Bouts T, Gacitua H, Hermes R, Schwarzenberger F. Cooling and freezing of Asian elephant (*Elephas maximus*) semen using directional freezing. *Theriogenology*. 2009;71(2):250-258.
 95. Sato Y, Yoshimoto-Kakoi K, Nakai M, Mizukami T, Tojo H. Production of functional sperm from porcine spermatogonial stem cells in xenografts. *Biol Reprod*. 2011;85(3):469-476.
 96. Shapiro B. How to clone a mammoth: the science of de-extinction. Princeton: Princeton University Press; 2017.
 97. Sharma S. Microfluidics in male reproduction: is ex vivo culture of primate testis tissue a future strategy for ART or toxicology research? *Mol Hum Reprod*. 2020;26:179-192.
 98. Shome R, Shome BR, Senani S, Saha SK, Padhi MK, Srivastava N. Isolation and characterization of *Brucella abortus* from bovines in Andamans. *Indian Vet J*. 1999;76(6):571-573.
 99. Srivastava SK, Shinde S, Singh SK, Mehrotra S, Verma MR, Singh AK, Srivastava N. Antisperm antibodies in repeat-breeding cows: frequency, detection and validation of threshold levels employing sperm immobilization, sperm agglutination and immunoperoxidase assay. *Reprod Domest Anim*. 2017;52(2):195-202.
 100. Stover J, Evans J, Dolensek EP. Inter species embryo transfer from the gaur to domestic Holstein. In: Proc Ann Meet Am Assoc Zoo Vet, Woodland Park Zoo, USA. 1981. p. 122-124.
 101. Swanson WF. Assisted reproductive technologies for managing genetic diversity and fertility in endangered species. *Theriogenology*. 2023;200:204-214.
 102. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663-676.
 103. Tamburrino L, Traini G, Marcellini A, Vignozzi L, Baldi E, Marchiani S. Cryopreservation of human spermatozoa: functional, molecular and clinical aspects. *Int J Mol Sci*. 2023;24(5):4656.
 104. Tetsuka M, Ogawa S, Yamaguchi M, Fukui Y. Development of ARTs for endangered species: experiences with large animals including elephants and marine mammals. *J Reprod Dev*. 2004;50(Suppl):121-125.
 105. Tharasanit T, Thuwanut P. Oocyte cryopreservation in domestic animals and humans: principles, techniques and updated outcomes. *Animals*. 2021;11:2949.
 106. Tharasanit T, Thuwanut P, Chatdarong K, Axné E. Production of kittens after intracytoplasmic sperm injection using sperm retrieved from cryopreserved testicular tissues in domestic cats. *Reprod Domest Anim*. 2012;47(Suppl 6):175-177.
 107. Thuwanut P. A case report concerning male gametes rescued from a Siamese Eld's deer (*Rucervus eldii siamensis*): post-thawed testicular and epididymal sperm quality and heterologous zona pellucida binding ability. *J Vet Med Sci*. 2013;75(1):123-125.
 108. Toyooka Y, Tsunekawa N, Akasu R, Noce T. Embryonic stem cells can form germ cells *in vitro*. *Proc Natl Acad Sci USA*. 2003;100:11457-11462.
 109. Trimeche A, Renard P, Tainturier D. A procedure for Poitou jackass sperm cryopreservation. *Theriogenology*. 1998;50:793-806.
 110. Vasconcelos ABM, Silva AM, Silva TF, Silva AR. Comparison of freezing protocols for cryopreservation of

- bovine epididymal spermatozoa. Cryobiology. 2013;66(2):111-114.
111. Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*. 1999;394(6691):369-374.
 112. Wani NA, Skidmore JA, Allen WR. Chemical activation of camel (*Camelus dromedarius*) oocytes following *in vitro* maturation. *Reprod Domest Anim*. 2017;52(3):390-397.
 113. Wildt DE, Pukazhenthil BS, Brown JL, Monfort SL, Howard JG, Roth TL. Spermatology and assisted reproduction in wildlife species. In: Andrade MCT, Andrade MCM, editors. *Spermatology*. Society of Reproduction and Fertility; 2010. p. 527-544.
 114. Wildt DE, Rall WF, Crister JK, Monfort SL, Seal US. Genome resource banks: 'living collections' for biodiversity conservation. *Bioscience*. 1997;47:689-698.
 115. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997;385(6619):810-813.
 116. Wisely SM, Santymire R, Livieri TM, Mueting SA, Howard J. A roadmap for 21st century genetic restoration: gene pool enrichment of the black-footed ferret. *Anim Conserv*. 2015;18(S1):77-81.
 117. Xin M, Siddique MAM, Dzyuba B, Cuevas-Urbe R, Shaliutina-Kolešová A, Linhart O. Progress and challenges of fish sperm vitrification: a mini review. *Theriogenology*. 2017;98:16-22.
 118. Yamanaka S. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell*. 2012;10:678-684.
 119. Yanez LZ, Camarillo DB. Microfluidic analysis of oocyte and embryo biomechanical properties to improve outcomes in assisted reproductive technologies. *Mol Hum Reprod*. 2017;23:235-247.
 120. Zhang L, Zhang M, Zhu S, Li C. Semen cryopreservation and artificial insemination in camels: a tool for genetic conservation. *Anim Reprod Sci*. 2017;182:115-123.

How to Cite This Article

Yadav L, Srivastava N, Kamothi DJ. Reproductive biotechnologies for conservation of endangered mammalian species. *International Journal of Veterinary Sciences and Animal Husbandry* 2026; 11(1): 403-411.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.