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REPRODUCTIVE BIOLOGY IN WILDLIFE CONSERVATION: RECENT ADVANCES, PERSISTENT CHALLENGES, AND KNOWLEDGE GAPS

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ABSTRACT

Reproductive science and assisted reproductive technologies are increasingly important components of wildlife conservation because they can help preserve genetic diversity, address reproductive failure, and connect ex situ management with in situ recovery. This review summarizes recent advances in wildlife reproductive science and examines the biological, technical, logistical, and ethical barriers that continue to limit routine conservation application. Major advances include non-invasive reproductive monitoring using biomarkers, improved sperm and tissue cryobiology, in vitro fertilization and embryo production in rhinoceroses, genome-resource banking, cloning from historically cryopreserved cells, and rapid development of spermatogonial stem-cell and induced pluripotent stem-cell technologies. Progress, however, remains uneven among taxa. Artificial insemination and semen cryopreservation are comparatively well developed in selected mammals, whereas female-germline preservation, embryo technologies in many non-model species, functional gamete production from stem cells, and assisted reproductive technologies in reptiles remain less mature. A central challenge is translating laboratory success into measurable conservation benefit. Reproductive interventions become conservation-relevant only when they improve genetic representation, demographic resilience, animal welfare, or population recovery. Future programmes should prioritize species-specific reproductive phenotyping, harmonized biobank metadata, multi-institutional validation, integration of biobanks with genomics and population modelling, and prospective ethical evaluation. Reproductive technologies should complement, rather than replace, habitat protection and natural breeding.

Keywords: Assisted Reproductive Technologies, Biodiversity, Conservation Breeding, Cryopreservation

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Introduction

Population persistence depends on more than the number of individuals remaining. Long-term viability also requires successful reproduction, the maintenance and transmission of genetic variation, appropriate age and sex structures, and the capacity to

produce offspring under variable ecological conditions. Reproductive failure may result from infertility, poor nutrition, endocrine disorders, disease, social incompatibility, reproductive aging, or the absence of genetically appropriate mates. In threatened species, these constraints are especially important because each breeding opportunity can have disproportionate

demographic and genetic value. Reproductive science therefore contributes to conservation in two complementary ways: by clarifying when and why animals reproduce and by enabling intervention when natural reproduction is inadequate or impractical. Because reproductive strategies vary widely among vertebrates, no single reproductive protocol can be expected to succeed across all species, including closely related taxa (Wildt et al. 2010).

Wildlife assisted reproductive technologies have largely been adapted from methods developed for cattle, horses, laboratory rodents, and humans; however, these methods do not transfer directly to non-domestic species. The progression from gamete collection to establishment of pregnancy may be influenced by species-specific differences in ovarian dynamics, sperm structure, gamete membrane composition, ovulatory mechanisms, seasonality, embryonic diapause, placentation, reproductive-tract anatomy, and responses to exogenous gonadotropins. Pukazhenthi and Wildt (2004) emphasized this principle early in the development of conservation reproductive technologies, and it remains a major constraint more than two decades later. Fundamental reproductive physiology is therefore not merely a prerequisite for biotechnology; it is the foundation of successful biotechnology. Nevertheless, substantial progress has been achieved in recent years. Semen, embryos, gonadal tissues, and somatic cells are now stored in conservation biobanks; repeatable oocyte recovery and in vitro embryo production have been achieved in white rhinoceros programmes; stem-cell and germline-preservation approaches have progressed from

conceptual work to experimental pipelines; and genetic rescue through cloning has been demonstrated using cells stored for decades. At the same time, investigators have increasingly recognized uncertainties related to cryptic species, developmental plasticity, epigenetic effects, protocol transferability, and the long-term fitness of offspring produced through intensive intervention (Holt and Comizzoli, 2026). These advances justify a contemporary reassessment that links reproductive biology and technological maturity to conservation decision-making rather than treating assisted reproduction as an end in itself.

Review approach and scope

This article presents a narrative review. Relevant literature was identified through PubMed and publisher databases, with emphasis on peer-reviewed English-language studies addressing wildlife reproductive physiology, reproductive monitoring, assisted reproduction, cryobiology, biobanking, cloning, and stem-cell technologies. Literature published from 2019 through August 2026 was prioritized, while selected earlier studies that remain foundational to current concepts and practice were retained. The review is not intended as a systematic inventory or meta-analysis. Instead, it covers mammals, birds, amphibians, and reptiles and integrates biological mechanisms, illustrative conservation case studies, major knowledge gaps, and priorities for knowledge translation. Examples were selected to represent major technology categories, important taxonomic contrasts, and barriers to translation rather than to generate pooled effect estimates.

Integrated reproductive-conservation pathway

Reproductive technologies create conservation value only when linked to species biology, genetics, welfare and population goals.

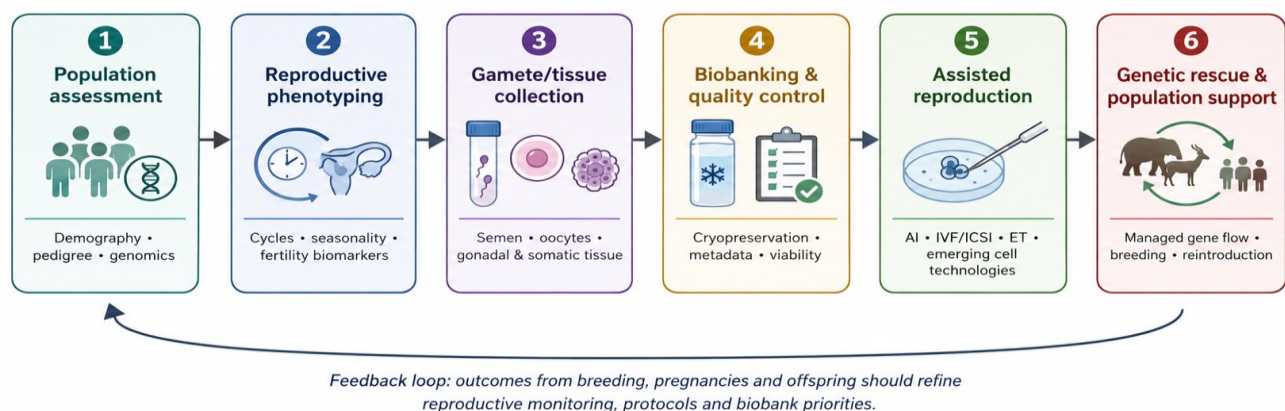


Figure 1. Integrated reproductive-conservation pathway. Original conceptual figure prepared for this review.

Reproductive biology as the foundation of conservation breeding

Species-specific reproductive phenotypes

A fundamental question in conservation breeding is deceptively simple: when are individuals reproductively competent? Answering this question may require longitudinal characterization of ovarian cycles, testicular activity, seasonality, sexual maturation, reproductive aging, and social or environmental modulation. Wild felids illustrate this complexity particularly well. Species, and even individuals within species, may differ in ovulatory pattern, ovarian cyclicity, and response to hormonal stimulation, while male fertility may be influenced by poor semen quality associated with small populations and reduced genetic diversity. Thongphakdee et al. (2020) reviewed reproductive research in wild felids and showed that approximately 15 years of work on domestic-cat physiology informed the development of artificial insemination, embryo production, and embryo transfer in wild species, rather than supporting the direct adoption of domestic-cat protocols.

The cheetah provides a clear example of how fundamental reproductive biology can guide management. Females may remain reproductively inactive for prolonged periods, while oocyte competence can remain relatively high across a broad age range; in contrast, age-associated uterine changes may progressively reduce the capacity to sustain pregnancy. Male cheetahs frequently produce ejaculates containing a high proportion of morphologically abnormal spermatozoa, but this characteristic does not translate directly into infertility. Crosier et al. (2022) demonstrated the value of integrating endocrine monitoring, ultrasonography, gamete assessment, and assisted breeding in ex situ cheetah management. For conservation purposes, such an integrated reproductive phenotype is more informative than any single measure, such as sperm motility or circulating hormone concentration.

Non-invasive reproductive monitoring

Repeated endocrine assessment in wildlife can be challenging because capture and restraint may induce stress, anesthesia may be required, and repeated handling may be impractical in free-ranging populations. Longitudinal reproductive monitoring can instead be achieved through repeated non-invasive collection of feces or urine, as well as hair, feathers, and other biological matrices. These approaches can identify ovarian cycles, pregnancy, reproductive suppression, and seasonal changes, particularly when combined with behavioral and environmental data. Edwards (2026) noted that the rapidly expanding range of non-invasive reproductive biomarkers represents one of the most promising opportunities for scaling reproductive monitoring across vertebrates. However, each assay must be biologically validated for the target species and sample matrix; cross-reactivity alone is insufficient to establish physiological relevance.

Natural breeding remains a benchmark, not a competitor

The availability of reproductive technologies should not create the impression that assisted reproduction is inherently superior to natural mating. In giant pandas, analysis of 21 years of breeding records showed that natural mating produced significantly more cubs than artificial insemination alone, highlighting the importance of behavioral compatibility, appropriate timing, and species-specific breeding management. Li et al. (2017) concluded that although artificial insemination can support specific genetic objectives, it should not replace management practices that facilitate natural reproduction. This principle is broadly applicable: assisted reproductive technologies are most justified when they address a defined constraint, such as geographic separation, genetic imbalance, reproductive disease, loss of a donor, or inability to mate, rather than when they are used simply because the technology is available.

Table 1. Reproductive features that influence conservation intervention

Feature	Why it matters	Conservation consequence	Representative taxa/examples
Seasonality and photoperiod	Determines timing of gametogenesis, estrus and ovulation.	Collection, hormonal stimulation and insemination must be scheduled within biologically responsive periods.	Many ungulates, carnivores, birds.
Ovulation mechanism	Species may be spontaneous, induced or show mixed patterns.	Hormonal induction and insemination timing require species-specific validation.	Felids, mustelids, camelids.
Sperm morphology and membrane biology	Affects collection, extension, cooling and cryosurvival.	Freezing media and cooling rates cannot be generalized across taxa.	Felids, wild ruminants, reptiles.

Embryonic strategy	Diapause, implantation timing and placentation vary markedly.	Embryo culture and recipient synchrony may become the limiting steps.	Mustelids, marsupials, diverse mammals.
Temperature-dependent development or sex determination	Environmental temperature can shape development and sex ratios.	Climate and incubation environment become reproductive-management variables.	Many reptiles; some amphibian contexts.
Reproductive ageing and pathology	Gamete competence and uterine/ovarian condition may diverge with age.	Preserve germplasm before irreversible reproductive senescence when possible.	Cheetahs, rhinoceroses, many long-lived mammals.

Cryopreservation and biobanking: preserving future options

Genome-resource banks provide an important link between animals living today and genetic material that may be required for future conservation. Spermatozoa, embryos, gonadal tissues, and somatic cells can be stored to extend the genetic contribution of founders, facilitate gene flow without transporting animals, preserve research material after an animal dies, and support technologies that may not yet be available. Bolton et al. (2022) described biobanking not merely as specimen storage but as infrastructure that can enable advanced reproductive technologies. The conservation value of a stored sample therefore depends on provenance, identity, health information, reproductive history, genetic representation, processing metadata, quality control, and the existence of a realistic pathway for future use.

Semen preservation is the most established form of wildlife reproductive biobanking, although performance varies substantially among taxa. A recent state-of-the-art review by Nagashima et al. (2025) documented major taxon-specific advances but also noted that cryopreserved germplasm remains underused in routine population management. Oocytes are particularly difficult to preserve because of their size, lipid content, sensitivity to chilling, and susceptibility to zona pellucida changes, while embryos and reproductive tissues require additional knowledge of developmental biology and recipient management. Accordingly, a 'frozen genetic resource' should not be assumed to represent readily recoverable future offspring. Biobanks should therefore evaluate not only storage success but also the functional reproductive potential of preserved material.

Male germline preservation

Sperm cryopreservation remains attractive because, in many species, semen can be collected from males at a lower biological cost than female gametes, and a single semen sample may contribute to the production of multiple offspring. In wild small ruminants,

cryoresistance is influenced by semen source, season, endocrine status, and molecular characteristics such as membrane water transport. Santiago-Moreno et al. (2023) highlighted the potential value of testes and epididymides for post-mortem genetic rescue, although post-thaw quality and fertility remain inconsistent. These findings reinforce an important conservation principle: technical survival through a freeze-thaw cycle does not necessarily indicate reproductive competence, and fertility endpoints should be assessed whenever feasible.

Female germline and gonadal tissues

In general, collection and cryopreservation of female gametes, including oocytes and embryos, are less developed than equivalent approaches for male gametes. Ovarian-tissue banking may partially address this imbalance because it can preserve large numbers of immature follicles from prepubertal animals, individuals that die unexpectedly, or animals that cannot undergo repeated oocyte collection. In Neotropical wildlife, Silva, Pereira and Comizzoli (2022) reviewed gonadal-tissue preservation and demonstrated its potential both for conservation and for research on gametogenesis. However, translating preserved tissue into viable offspring may require tissue culture, grafting, in vitro maturation, and embryo technologies that remain highly species specific.

Advances in human fertility preservation also provide useful comparative opportunities for wildlife conservation. Silva et al. (2025) noted that ovarian- and testicular-tissue cryopreservation, culture, and grafting share several underlying mechanisms across humans and wildlife, although important anatomical and physiological differences require substantial adaptation. Such cross-disciplinary transfer is particularly valuable for rare species, for which researchers may not have sufficient access to develop complex methods *de novo*. Domestic and biomedical models should therefore be used as hypothesis-generating platforms rather than as substitutes for validation in the target species.

Qualitative readiness of reproductive technologies across wildlife taxa

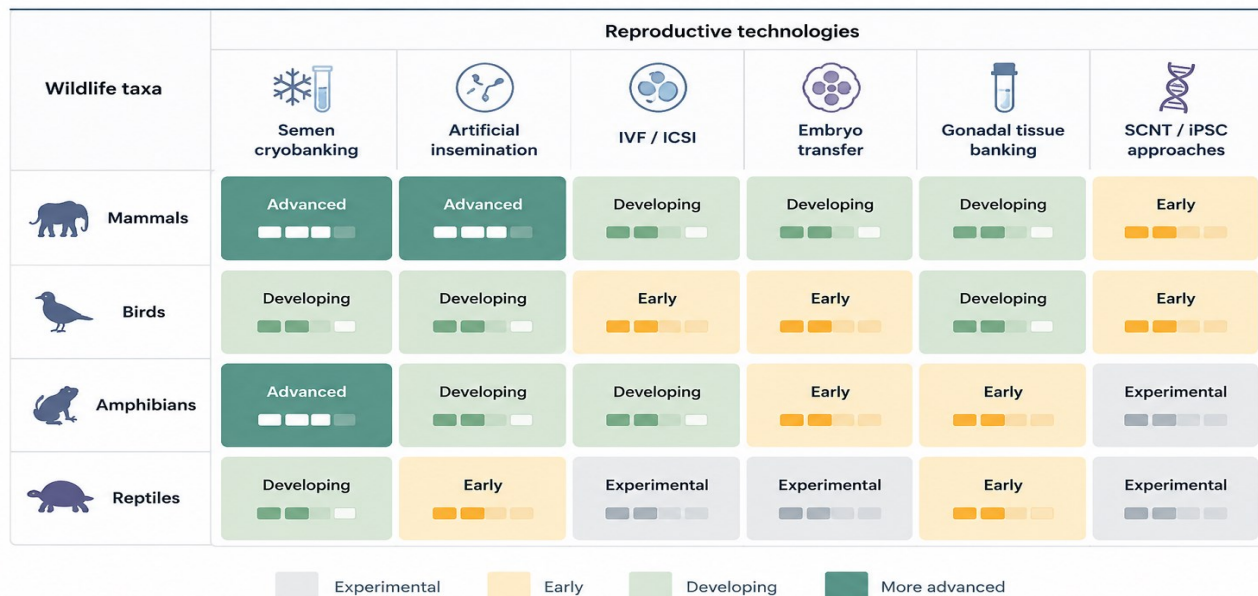


Figure 2. Qualitative readiness of selected reproductive technologies across major vertebrate groups. This is an author-derived synthesis, not a quantitative meta-analysis.

Assisted reproductive technologies: from artificial insemination to embryo transfer

Artificial insemination

Artificial insemination is among the oldest assisted reproductive technologies and is most likely to progress from research to routine management when semen collection, semen preservation, and the timing of ovulation are well characterized. Potential conservation benefits include reducing the need to transport animals, facilitating planned gene flow among institutions, using stored semen from geographically distant or deceased males, and overcoming behavioral incompatibility. Nevertheless, even among mammals, reliable success has been achieved in only selected species. After decades of work with wild felids, Swanson (2023) concluded that major constraints include incomplete knowledge of reproductive biology, limited access to research animals, institutional restrictions, and a tendency to adopt complex methods before simpler approaches have been adequately validated. This mismatch between technological ambition and biological understanding remains an avoidable cause of failure.

Oocyte recovery, in vitro fertilization and intracytoplasmic sperm injection

In vitro fertilization and intracytoplasmic sperm injection may be particularly useful when sperm numbers are low, donor animals are unable to mate, or only limited quantities of genetically valuable gametes are available. The northern white rhinoceros is the most prominent example of this strategy. Hildebrandt et al. (2023) reported a programme involving repeated ovum

pick-up, in vitro maturation, and in vitro fertilization to produce viable white-rhinoceros blastocysts. Importantly, this work demonstrates that advanced outcomes can become feasible when species-specific anatomy, anesthesia, ovarian stimulation, oocyte retrieval, laboratory embryology, and ethical oversight are developed together over many years.

Embryo transfer and recipient biology

Embryo production is only one stage in the generation of viable offspring. Recipient physiology is central to advanced assisted reproductive technologies and may differ substantially from domestic-animal models. Successful conservation outcomes require appropriate implantation, pregnancy maintenance, parturition, and production of healthy offspring, as well as adequate luteal support, uterine health, placentation, and fetal development. Reviewing approximately four decades of wildlife assisted reproduction, Mastromonaco (2024) concluded that the most substantial advances have occurred when research institutions, zoological collections, veterinary practitioners, and population managers have worked toward shared conservation objectives. Embryo transfer should therefore be regarded as a systems-level problem in which laboratory competence and recipient competence must be optimized simultaneously.

Species with stronger translational potential

Some taxa have greater potential for translation because closely related domestic species have already been validated as reproductive models. Bison provide an example in which knowledge from cattle has supported estrus synchronization, gamete handling,

embryo production, and cryopreservation. Acevedo and Barfield (2023) argued that these approaches can facilitate genetic exchange while reducing biosecurity risks and transport requirements in conservation herds. However, even closely related species can differ in

reproductive seasonality, follicular dynamics, and treatment responses. Domestic models therefore reduce, but do not eliminate, the need for species-specific validation.

Table 2. Major reproductive technologies in wildlife conservation

Technology	Primary conservation use	Strengths	Main limitations	Current position
Non-invasive hormone/biomarker monitoring	Cycle, pregnancy and fertility monitoring	Repeatable; low disturbance; useful in field or zoo settings	Requires species/matrix validation; metabolites may lag physiology	Widely useful and expanding
Semen collection and cryopreservation	Preserve and transport paternal genetics	Relatively efficient storage; can support artificial insemination and in vitro fertilization	Cryosensitivity; semen collection can require anesthesia	Most mature wildlife germline technology
Artificial insemination	Targeted gene flow without moving animals	Can use fresh or frozen semen; relatively simple conceptually	Timing, insemination site and ovulation biology are species specific	Operational in selected mammals; limited elsewhere
Oocyte recovery, in vitro fertilization, and intracytoplasmic sperm injection	Embryo production from valuable or non-breeding females	Efficient use of scarce sperm; bypasses some mating barriers	Oocyte competence, culture media and embryo development vary	High-value but restricted to specialized programs
Embryo transfer	Produce offspring from ex situ embryos	Separates genetic parents from gestational carrier	Recipient synchrony, uterine biology, pregnancy monitoring	Developing; few wildlife species have robust protocols
Ovarian/testicular tissue banking	Preserve immature germ cells and post-mortem genetics	Captures many germ cells; can include immature donors	In vitro gametogenesis or grafting is often required	Rapidly developing
Somatic cell nuclear transfer / cloning	Reintroduce underrepresented genomes from somatic cells	Can recover alleles from historical biobanks	Low efficiency; epigenetic/reproductive risks; surrogate needs	Demonstrated in select species, not routine
Induced pluripotent stem-cell / stem-cell gametogenesis	Potential future generation of germ cells/embryos	Could expand use of somatic-cell archives	Differentiation fidelity, imprinting, safety and regulation	Experimental

Emerging technologies and genetic rescue

Cloning and somatic cell nuclear transfer

Somatic cell nuclear transfer differs from conventional assisted reproduction because it can reconstruct an embryo using the genome of a stored somatic cell. This creates a distinctive opportunity to use historical cell banks for conservation. Swegen et al. (2023) reviewed cloning approaches and concluded that somatic cell nuclear transfer may be relevant to selected genetic-rescue scenarios but remains constrained by low efficiency, the need for suitable oocytes, surrogate compatibility, and risks of developmental abnormalities. These limitations restrict its routine use in breeding programmes. Its strongest conservation

justification is therefore the recovery of genetic variation that is absent or severely underrepresented in the living population.

This selective role has recently been illustrated in Przewalski's horse. Novak et al. (2025) described cloning from historically cryopreserved cells representing an underrepresented lineage, with the aim of increasing representation of rare genetic material in the contemporary population. This case moves long-term somatic-cell banking from a theoretical possibility toward a practical genetic-management option. It also emphasizes an important planning principle: samples collected and stored decades earlier may enable

conservation interventions that were not technically possible at the time of collection.

Spermatogonial stem cells and testicular germline rescue

Spermatogonial stem cells offer another approach for preserving and potentially propagating male genetic material. Testicular tissue can be collected from immature or adult animals and, in principle, used for transplantation, grafting, or in vitro culture to generate spermatozoa. Damyanova et al. (2024) reviewed the potential transfer of methods developed for human fertility preservation to wildlife and highlighted major gaps in the characterization of spermatogonial stem-cell markers, niche biology, and differentiation pathways in most non-model species. In the near term, the most practical strategy may therefore be to bank viable testicular tissue while methods for subsequent recovery and use continue to be developed in parallel.

Induced pluripotent stem cells and synthetic reproductive pathways

Somatic cells may provide a renewable source for pluripotent cell lines generated through induced pluripotent stem-cell technology, with potential applications in embryo models and future germline differentiation. Lou et al. (2025) summarized rapid progress in induced pluripotent stem cells derived from endangered animals, including non-integrating reprogramming methods and efforts to direct these cells toward germline lineages. This approach has considerable conservation potential because somatic cells are often easier to collect and bank than mature gametes. However, substantial challenges remain, including reliable germline differentiation, genomic stability, epigenetic fidelity, correct imprinting, and developmental competence. Reproductive applications based on induced pluripotent stem cells should therefore be considered a long-term research investment rather than a current population-management tool.

Comparative progress beyond mammals Amphibians

Hormonal induction, sperm cryopreservation, and artificial fertilization have been integrated into amphibian conservation research, making amphibians an important test group for these approaches. Many amphibian species face urgent conservation threats, and some reproductive technologies are comparatively inexpensive to apply relative to those required for large

mammals. Silla and Byrne (2019) summarized successful hormonal therapies, gamete-storage methods, and fertilization approaches while emphasizing that technical development must be followed by practical adoption in conservation breeding. Increasing attention is now being given to integrated reproduction-biobanking-conservation systems rather than isolated procedures, and both technical capacity and geographic equity are essential if these technologies are to reach regions with the greatest amphibian diversity and conservation need (Browne et al., 2024).

Reptiles

Assisted reproductive technologies in reptiles remain less developed than those in mammals and many amphibians. Protocol development is complicated by diverse reproductive anatomy, seasonal cycles, challenges in semen collection and sperm storage, prolonged sperm retention within the female reproductive tract, and variation in oviparous and viviparous reproductive modes. Perry and Mitchell (2022) reviewed advances in artificial insemination, hormonal control, gamete collection, and genome conservation while identifying major limitations in embryo manipulation, intracytoplasmic sperm injection, and cloning. Similarly, Clulow and Clulow (2016) emphasized that amphibians and reptiles require distinct technical solutions because of fundamental differences in reproductive biology and evolutionary history. Progress in these groups should therefore be viewed as parallel, taxon-specific development rather than as a linear extension of mammalian methods.

Neotropical wildlife and underrepresented taxa

Geographic and taxonomic bias remains a major limitation in conservation reproductive science. Detailed reproductive knowledge is disproportionately derived from charismatic species maintained in well-resourced institutions. Silva et al. (2026) reviewed recent advances in assisted reproductive technologies for Neotropical mammals and noted both scientific progress and limited large-scale implementation in conservation practice. They emphasized the need for regional biobanks, cautious use of domestic or model relatives, and sustained capacity development in biodiversity-rich countries. Without such investment, technological development risks becoming concentrated on species that are easiest to study rather than those with the greatest conservation need.

Table 3. Representative conservation case studies and lessons

Case	Reproductive tool	What has been achieved	Key lesson
White rhinoceros	Ovum pick-up, in vitro maturation, in vitro fertilization / intracytoplasmic sperm injection, embryo transfer	Viable embryos and proof-of-concept transfer pathway	Long-term investment in species-specific biology can make advanced assisted reproductive technologies feasible.

Przewalski's horse	Somatic cell nuclear transfer from a historical cell bank	Cloned individuals from historically cryopreserved cells	Somatic-cell archives can restore representation of lost lineages.
Cheetah	Endocrine monitoring, semen cryopreservation, artificial insemination, and embryo research	Improved reproductive management and assisted-breeding options	Reproductive ageing and uterine health may constrain success more than oocyte quality.
Giant panda	Natural mating and artificial insemination	Long-term breeding records enable evidence-based comparison of methods	Natural mating can outperform artificial insemination and should remain the baseline when feasible.
Bison	Synchronization, artificial insemination, embryo technologies, and biobanking	Domestic-bovine knowledge accelerates development of conservation assisted reproductive technologies	Close domestic models are valuable but still require wildlife validation.
Threatened amphibians	Hormonal induction, sperm banking, artificial fertilization	Operational protocols exist for multiple species	The remaining gap is implementation capacity and linkage to recovery programs.

Biological and technical challenges that still limit translation

Wildlife reproductive studies frequently involve sample sizes that would be considered inadequate in domestic-animal or biomedical research. Endangered species may have very few reproductively available individuals, studies are often opportunistic, and ethical or husbandry constraints can prevent experimental replication. Consequently, estimated treatment effects may carry substantial uncertainty, and protocol optimization may require long periods. Comizzoli (2015) argued that the limited contribution of fertility-preservation technologies to species conservation reflects not only technological barriers but also deficits in biological knowledge, facilities, expertise, and funding. These constraints can be as important as the development of a new protocol, making collaborative standardization and data sharing essential components of progress.

Cryobiological diversity

Cellular sensitivity to cooling and freezing is influenced by lipid composition, cell size, membrane permeability, osmotic tolerance, and developmental stage. Consequently, a cryoprotectant concentration that is optimal for one species may be ineffective or harmful in another. Multicellular organization, intracellular lipid content, and diffusion gradients create additional challenges for oocytes, embryos, and tissues. Pukazhenthi et al. (2006) emphasized both the potential of emerging technologies for endangered species and the central role of fundamental biology in determining their effectiveness. Despite the range of approaches available in modern cryobiology, species-specific optimization remains unavoidable.

Reproductive ageing, disease and hidden pathology

Conservation programmes may identify genetically valuable individuals while reproductive function declines unnoticed with age. Long-lived animals in captivity can remain demographically important after their reproductive capacity has diminished substantially. This is one reason proactive biobanking is preferable to relying on emergency salvage after a reproductive crisis has occurred. Comizzoli and Holt (2019) emphasized the importance of identifying such latent physiological constraints before they become irreversible and limit opportunities for reproductive intervention in rare species. Reproductive-health monitoring should therefore be integrated into genetic management rather than treated solely as an element of veterinary care.

Laboratory endpoints to conservation endpoints

Laboratory endpoints such as sperm motility, membrane integrity, cleavage rate, and blastocyst formation are useful proxies, but conservation outcomes occur at the population level. Relevant outcomes include retained heterozygosity, representation of founders, reduced inbreeding, viable pregnancies, healthy offspring, reproductive competence of descendants, and, where appropriate, successful reinforcement or reintroduction. Holt and Comizzoli (2022) emphasized that reproductive science has important value but also clear limitations when it is considered outside the ecological and demographic context of the target species. Reproductive interventions should therefore be selected according to the conservation outcome that a programme is intended to achieve.

Ethics, welfare, governance and social legitimacy

Anesthesia, repeated hormonal treatment, surgery, transrectal procedures, embryo manipulation, use of surrogate females, and intensive veterinary care may all form part of assisted reproductive technologies. Such procedures may be ethically justified when they support high-priority conservation objectives, but technical feasibility does not by itself establish ethical acceptability. Biasetti et al. (2022) developed an ethical-analysis framework for ovum pick-up in white rhinoceroses that considered animal welfare, conservation value, procedural quality, and stakeholder interests. Prospective ethical assessment, rather than evaluation only after a study has been completed, can influence study design, stopping criteria, procedure frequency, and the selection of alternatives from the outset.

Ethical evaluation also has a strategic dimension. Expensive reproductive interventions must be considered alongside alternative uses of conservation resources, including habitat management, disease control, anti-poaching measures, community engagement, and less complex breeding strategies. The relevant comparison is therefore between a proposed intervention and the best alternative use of the same conservation resources. This consideration is especially important for functionally extinct or extremely small populations, in which ambitious reproductive technologies may generate substantial scientific knowledge without guaranteeing population recovery. Clear objectives, prospective welfare assessment, and predefined criteria for success and discontinuation are essential for maintaining scientific and public credibility.

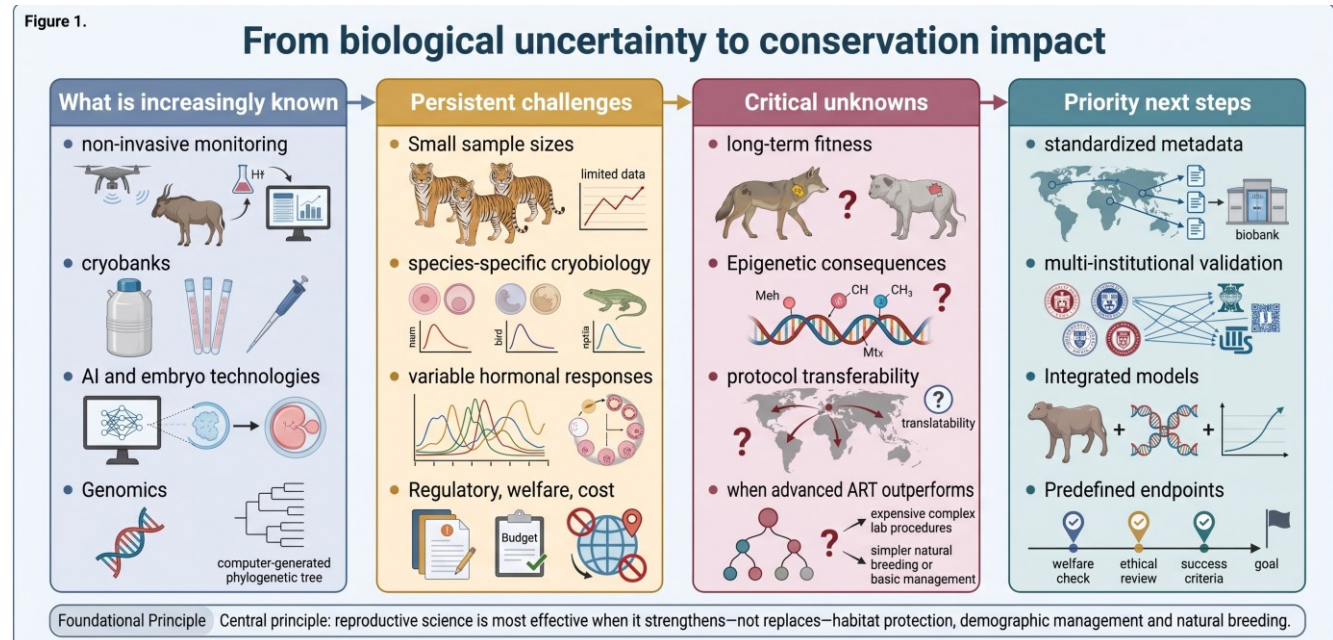


Figure 3. Knowledge landscape and priority actions for wildlife reproductive science.

Future directions

The next phase of wildlife reproductive science should place less emphasis on isolated technical milestones and greater emphasis on reproducible conservation systems. Several priorities emerge from the current literature.

- 1. Build species-specific reproductive baselines before crisis points.** Routine endocrine, ultrasonographic, semen, and reproductive-health data should be collected across life stages so that interventions are not developed only after fertility has already declined.
- 2. Bank broadly, but bank intelligently.** Cryobanks should prioritize genetically informative donors and include standardized metadata on identity, health, provenance, processing, and quality control. Somatic and gonadal tissues should complement, rather than replace, gamete banking.

- 3. Integrate genomics with reproductive decision-making.** Genomic data can help identify underrepresented lineages, confirm taxonomic identity, reduce inadvertent inbreeding, and determine which stored samples have the greatest potential conservation value.
- 4. Validate technologies across institutions.** A protocol that succeeds in one laboratory with a small number of animals should be independently reproduced before it is considered ready for management use. Shared standard operating procedures and common outcome measures are essential.
- 5. Treat welfare and ethics as design variables.** Procedural burden, anesthesia frequency, hormonal exposure, surrogate welfare, and opportunity costs

should be evaluated prospectively, with explicit thresholds and clearly defined alternatives.

- 6. Measure descendant and population-level outcomes.** Follow-up should include neonatal survival, growth, fertility, health, and, where relevant, performance after reintroduction. Population models should determine whether assisted reproductive technologies materially improve genetic or demographic outcomes.

7. Invest in underrepresented taxa and regions.

Technical capacity should extend beyond a small number of charismatic mammals and high-income institutions. Regional biobanks and training networks will determine whether reproductive technologies contribute to global biodiversity conservation rather than to a limited subset of species.

Table 4. Knowledge gaps and research priorities

Gap	Why it matters	Recommended research	Conservation decision enabled
Sparse baseline reproductive physiology	Poor timing and inappropriate protocols lead to failed interventions.	Longitudinal non-invasive endocrinology, imaging and fertility phenotyping.	Choose natural breeding, artificial insemination, or advanced assisted reproductive technologies at the appropriate reproductive stage.
Unvalidated cryopreservation protocols	Stored material may have little practical reproductive value.	Species-specific cryoprotectant, cooling and post-thaw fertility studies.	Prioritize samples that can realistically be used for gene flow.
Weak female-germline preservation	Maternal genetic contribution is harder to bank than paternal contribution.	Oocyte vitrification, ovarian-tissue culture, in vitro maturation, and embryo-competence studies.	Balance representation of male and female founders in biobanks.
Limited recipient/surrogate knowledge	Embryos cannot contribute to populations without competent gestation.	Cycle synchronization, luteal physiology, uterine health and pregnancy monitoring.	Determine whether embryo transfer is biologically and ethically feasible.
Unknown long-term fitness of offspring produced through assisted reproductive technologies	Birth alone does not prove conservation value.	Lifetime health, reproductive and behavioral follow-up; molecular/epigenetic monitoring.	Assess whether interventions improve sustainable population fitness.
Disconnected reproductive and population models	Technically successful offspring may be genetically redundant.	Link pedigrees and genomics with demographic simulations and probabilities of success for assisted reproductive technologies.	Allocate resources to interventions with greatest marginal conservation benefit.

Conclusion

Reproductive biology has become an integral component of conservation planning for some threatened species and an important supporting discipline for others. The greatest advances have resulted not from technology alone, but from coordinated systems in which reproductive physiology, veterinary care, genomics, biobanking, and population management inform one another. Semen cryopreservation and artificial insemination are increasingly applied in selected mammals and amphibians; species-specific embryo technologies continue to advance; gonadal-tissue preservation expands the range of life stages that can be banked; and

cloning and stem-cell technologies are extending the potential use of somatic-cell archives for recovery of genetic variation. Nevertheless, substantial uncertainties remain, including small sample sizes, cryobiological diversity, recipient physiology, reproductive aging, welfare constraints, regulatory barriers, and limited knowledge of the long-term fitness of offspring produced through assisted reproduction. The most productive future direction is therefore not technological escalation, but the strategic selection of the least intrusive effective intervention for a clearly defined conservation objective. Habitat protection, maintenance of healthy wild populations, and natural reproduction remain the foundation of conservation.

Reproductive technologies are most valuable when they preserve future options, restore otherwise lost genetic

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lineages, and address constraints that cannot be resolved through conventional management alone.

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