Review

Assisted Reproductive Technologies for Livestock Biobanking

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ABSTRACT

Conservation of livestock genetic resources is essential for biodiversity, sustainable agriculture, and future breeding programs. Cryobanking and assisted reproductive technologies (ARTs) have emerged as vital tools for preserving and utilizing valuable or endangered livestock species. Sperm and oocyte banking allow long term storage of individual genetic material, whereas embryo banking through *in vitro* embryo production (IVEP), including ovum pick-up (OPU), *in vitro* fertilization (IVF), and intra-cytoplasmic sperm injection (ICSI) preserves the complete parental genome. Somatic cell banking combined with somatic cell nuclear transfer (SCNT) and interspecies SCNT (iSCNT) further enhances conservation by allowing the generation of cloned animals from cryopreserved cells, even after the death, enabling restoration of lost genetics. Despite progress, challenges remain, including technical strength, infrastructure, low embryo development rates, cryosensitivity, and SCNT inefficiencies. Advances in cryopreservation techniques, epigenetic reprogramming, and automation are improving the success and applicability of these technologies. In conclusion, integration of multiple ARTs with national and global gene banks provides a comprehensive strategy for maintaining genetic diversity and supporting livestock sustainability.

Keywords: Biobanking, Assisted Reproductive Technologies, IVEP, SCNT, Gene Banks

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INTRODUCTION

The loss of animal genetic resources is accelerating due to human driven environmental changes, occurring at rates 1,000-10,000 times faster than natural extinction processes (Ceballos and Ehrlich, 2018; Turvey and Crees, 2019). According to current IUCN assessments, 41% of amphibians, 26% of mammals, and 12% of birds are threatened with extinction, representing the most serious biodiversity crisis in the past 65 million years (IUCN, 2021). In livestock, the major factors contributing to genetic erosion include the widespread introduction of exotic breeds (64%), indiscriminate crossbreeding (29%), production intensification (29%), and shrinking habitats (21%) (FAO, 2023). To counter this, both *in situ* (on-farm and habitat-based conservation) and *ex situ* (outside the natural habitat) strategies are essential. *Ex situ* measures such as captive breeding and biobanking are increasingly recognized as critical (Comizzoli *et al.,* 2019; Andrabi and Maxwell, 2007). Assisted reproductive technologies (ARTs) play a key role in this process. Conventional ARTs include semen collection, artificial insemination (AI), ovum pick-up (OPU), *in vitro* fertilization (IVF), and embryo transfer (ET). Advanced ARTs, including somatic cell nuclear transfer (SCNT), stem cell based gametogenesis, embryo/sperm sexing, and cryobanking, provide powerful options (Paiva *et al.,* 2016).While ARTs are valuable, they complement natural breeding and husbandry, with gene banks remaining essential for long term biodiversity restoration (Greggor *et al.,* 2018; Blackburn *et al.,* 2023).This review focuses on the key ARTs used for conserving and reviving endangered, vulnerable and extinct animal genetic resources (AnGR).

Biobanking

The rapid rise in species extinctions makes it impractical to design separate conservation programs for every threatened animal. Cryopreservation has emerged as a cornerstone technology in reproductive biotechnology because it enables indefinite storage of germplasm (sperm, oocytes, embryos, and cells), thereby creating long-term genetic repositories. These gene banks support breed conservation, safeguard genetic diversity, expand the genetic base, facilitate new breed development, and provide valuable resources for research (Zomerdijk *et al.,* 2020). In essence, biobanking secures the genetic past to strengthen the future of biodiversity, protecting against genetic erosion, buffering populations from disease outbreaks, and enabling restoration through reintroduction of valuable genetics (Blackburn *et al.,* 2023). However, preserving live cells requires highly specialized expertise, tailored cryopreservation protocols for each species and cell type, optimized media or extenders, sterile laboratory conditions, and effective cryoprotectants. According to FAO (2015), gene banks are the principal tool for *in vitro* conservation of

AnGRs, with facilities now operational in 128 countries worldwide. A wide range of biological materials has already been preserved including semen, embryos, oocytes, somatic cells, iPS cells, embryonic stem cells, reproductive tissues, organoids, blood, primordial germ cells (PGCs), serum, and DNA across livestock, poultry, and wild species (Woelders *et al.,* 2012; Engdawork *et al.,* 2024). Globally, bio-repositories have been established to house diverse cell sources for future use (Paiva *et al.,* 2016; Ren *et al.,* 2025). Among these, four commonly used banking approaches (Fig. 1) in livestock species include:

1. Sperm Banking – The most widely used approach, enabling efficient and cost-effective long term storage of semen. It facilitates AI, crossbreeding, breed conservation, and genetic introgression programs.
2. Oocyte Banking – Though technically more challenging due to sensitivity of oocytes to cryoinjury, this approach preserves female germplasm and supports IVF, ICSI, and advanced reproductive programs.
3. Embryo Banking – Allows conservation of combined paternal and maternal genetics in a single embryo. Embryo transfer from cryopreserved embryos is routinely applied for breed regeneration and germplasm distribution.
4. Somatic Cell Banking – Stores fibroblasts and other somatic cells that can later be reprogrammed for SCNT (cloning) or induced into pluripotent stem cells (iPSCs). This expands conservation possibilities beyond gametes and embryos, providing backup for endangered or rare breeds.
5. These biobanking strategies form the backbone of global efforts to conserve livestock biodiversity, ensure food security, and provide genetic resources for future generations.

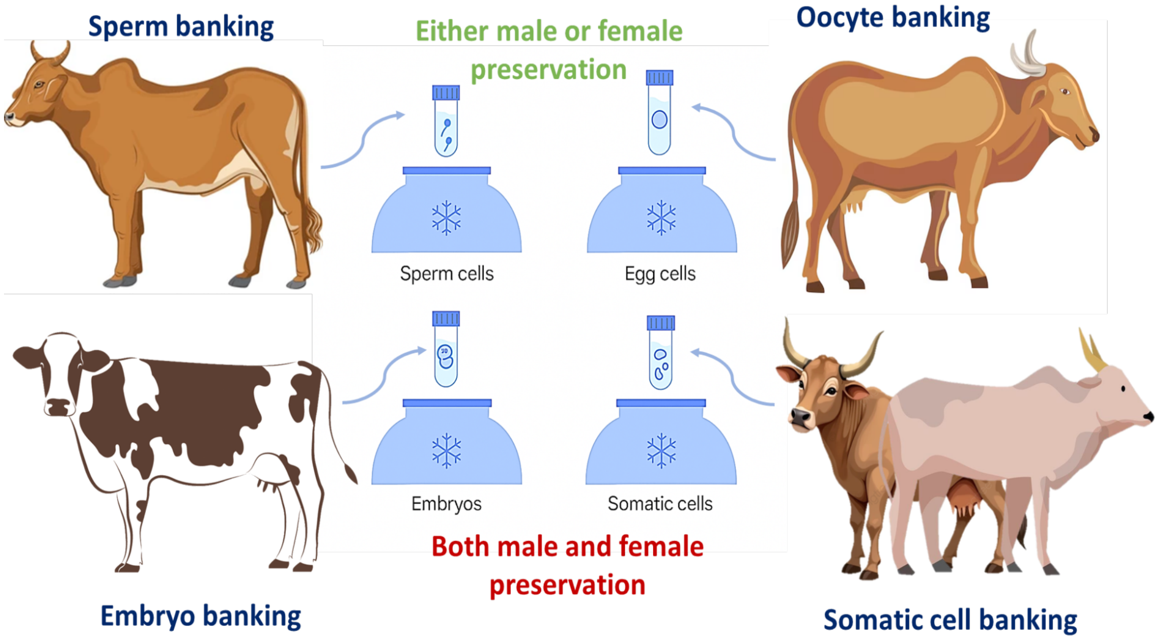

Fig. 1: *Most commonly used biobanking strategies in livestock species. A) Sperm Banking: Semen cryopreservation is a routine practice in livestock for artificial insemination and genetic improvement. It enables long term storage and global exchange of male germplasm at −196°C. B) Oocyte Banking: Oocyte cryopreservation preserves female germplasm for assisted reproductive technologies and genetic conservation. C) Embryo Banking: Embryo cryopreservation allows conservation of both maternal and paternal genomes in livestock species. It supports breed improvement, germplasm exchange, and preservation of endangered breeds. D) Somatic Cell Banking: Somatic cells such as fibroblasts can be cryopreserved for use through somatic cell nuclear transfer. This serves as a backup strategy when gametes are unavailable for conserving rare livestock genetics.*

Sperm Banking and Artificial Insemination

Sperm banking, through cryopreservation, and artificial insemination (AI) are two interlinked reproductive biotechnologies that have transformed animal breeding and genetic conservation. AI is the most widely applied ART across domestic livestock and certain wild species, valued for its high efficiency, cost-effectiveness, and biosecurity advantages. It enables rapid dissemination of genetic material on a large scale, making it a foundation of modern breeding programs as well as a key tool in *ex situ* conservation (Foote, 2002; Holt and Lloyd, 2009).

Sperm banking, which is achieved by cryopreserving semen at -196 °C in liquid nitrogen, ensures long-term storage and facilitates global germplasm exchange under standardized conditions. Advances in semen extenders, novel cryoprotectants (such as glycerol and egg yolk substitutes), and optimized thawing methods have significantly improved post-thaw sperm motility, viability, and fertility outcomes across livestock species (Engdawork *et al.,* 2024; Murray and Gibson, 2022). Together, sperm banking and AI provide a practical framework to safeguard genetic diversity and restore traits that might otherwise be lost in breeding populations (Seidel, 2014; Blackburn, 2023). By integrating sperm banking with AI, gene banks worldwide are ensuring that preserved semen can be effectively used in future breeding programs, thereby linking conservation with practical applications in livestock improvement (Fickel *et al.,* 2007).

Globally, AI and sperm banking are embedded within genetic improvement and biodiversity conservation frameworks. For example, the USDA National Animal Germplasm Program (NAGP) employs sperm banking to secure genetic resources and uses AI to reintroduce diversity into both commercial and heritage breeds (Blackburn, 2023). Similarly, in India the National Gene Bank located at ICAR-NBAGR, Karnal, Haryana, maintains cryopreserved semen from multiple livestock breeds, ensuring the long term availability of genetic resources for breed rescue and restoration programs (ICAR-NBAGR, 2023).

Oocytes or Embryo Banking and In-Vitro Embryo Production

Unlike sperm or oocyte banking, which preserves genetics of only one parent (n), embryo banking captures both maternal and paternal genetic material (2n), allowing genetic improvement and better conservation of valuable or endangered animals (Hasler, 2014). Advances in *in-vitro* embryo production (IVEP) have made embryo banking more efficient, especially when combined with ovum pick-up and *in vitro* fertilization (OPU-IVF) and intra cytoplasmic sperm injection (ICSI). OPU allows repeated collection of oocytes from live endangered females. These oocytes are matured in the lab, fertilized either by conventional IVF or by ICSI, and developed into embryos at the blastocyst stage. The embryos can then be transferred into recipient females or cryopreserved in gene banks for long-term storage (Velazquez, 2008).

Since the first IVEP derived calf was born in 1981, this technology has been used widely to multiply animals, protect rare breeds, and accelerate livestock improvement (Brackett *et al.,* 1982; Galli and Lazzari, 2008). IVF involves incubating mature oocytes with prepared sperm in culture medium, allowing natural fertilization. This method works best when oocytes and sperm are abundant and of good quality. Fertilized oocytes are then cultured until the blastocyst stage, ready for transfer into recipient females or cryopreserved for future use.

ICSI, on the other hand, is used when conventional IVF may fail, such as in species with poor fertilization rates, when sperm numbers are limited, or when frozen gametes are used. In ICSI, a single spermatozoa is directly injected into the oocyte cytoplasm. This technique is especially useful for conservation purposes, including interspecific fertilization, where oocytes from closely related species are used; for example, injecting cheetah or leopard sperm into domestic cat oocytes (Moro *et al.,* 2014). Mouse oocytes have also been used to test freeze-dried sperm from species like chimpanzees, giraffes, and jaguars (Kaneko *et al.,* 2014).

Despite some species specific challenges, such as low oocyte recovery or embryo cryosensitivity, ongoing improvements in culture media, cryopreservation methods, ICSI automation, and embryo biology regulation continue to increase the efficiency of IVEP. Importantly, embryo banking complements sperm and oocyte storage by preserving the full genetic makeup of livestock, including unique alleles and maternal lineages that semen banking alone cannot conserve. Therefore, IVEP based embryo banking, together with IVF and ICSI techniques, is not only a tool for productivity but also a strategic approach to protect genetic diversity, support sustainable agriculture, and conserve endangered livestock.

Somatic Cell Banking and Somatic Cell Nuclear Transfer

Since the first successful cloning of mammals in 1997 (Wilmut *et al.,* 1997), SCNT has been explored as a valuable tool for conserving endangered and rare species. In SCNT, the nucleus from a somatic cell of a valuable or threatened animal is transferred into an enucleated oocyte to create a cloned embryo, which can then be cultured to the blastocyst stage before being transferred to a surrogate mother or cryopreserved for future use (Iqbal *et al.,* 2021; Andrabi and Maxwell, 2007). SCNT has been successfully applied to clone farm animals with desirable traits, safeguard rare cattle breeds such as Enderby, Zhangmu, and Apeijiaza (Wells *et al.,* 1998; Backus, 2006; Qinging, 2024), and restoration of genetic from cryopreserved somatic cells of deceased males (Selokar *et al.,* 2014). Conservation successes include the birth of a cloned black-footed ferret in 2024, which successfully reproduced in a breeding program (Novak *et al.,* 2024), as well as cloning of Przewalski's horse, demonstrating SCNT's potential in biodiversity programs.

Somatic cell banking is a key component of SCNT based conservation. It involves collecting and cryopreserving somatic cells, such as skin fibroblasts, blood cells, or other tissue types, to create a long term genetic repository. These preserved cells can be revived and used for SCNT at any time, enabling the reproduction of animals even after the donor has died. Somatic cell banking safeguards valuable alleles and maternal or paternal lineages, helping maintain genetic diversity in livestock and endangered species. This approach also provides flexibility for research, breeding programs, and conservation initiatives without relying on living animals. In line with this, ICAR-NBAGR has initiated the banking of skin-derived cells from Indian livestock species, ensuring long term preservation of genetic resources for future breed rescue and restoration programs (ICAR-NBAGR, 2023; Nagarajan *et al.*, 2023; Prasad *et al.,* 2023).

To overcome the limitation of scarce oocytes, interspecies SCNT (iSCNT) has been developed, in which the nucleus from one species is transferred into the enucleated oocyte of a closely related species. This technique has successfully produced offspring in several cases, including wild buffalo with domestic buffalo oocytes, gaur with cattle oocytes, African wildcat with domestic cat oocytes, gray wolf with dog oocytes, Bactrian camel with dromedary oocytes, and sand cat with domestic cat oocytes (Priya *et al.,* 2014; Gomez *et al.,* 2004; Oh *et al.,* 2008; Wani *et al.,* 2017). Despite its promise, SCNT and iSCNT face challenges such as low efficiency, developmental abnormalities caused by incomplete epigenetic reprogramming (Nguyen *et al.,* 2022; Pankammoon *et al.,* 2025). Nevertheless, with ongoing improvements in somatic cell preservation, donor cell quality, reprogramming techniques, and standardized laboratory protocols, SCNT combined with somatic cell banking offers a powerful approach to safeguard endangered species and rare genetic lines, supporting the survival of threatened populations across generations while complementing broader conservation and biodiversity efforts (Novak *et al.,* 2025; Cowl *et al.,* 2024).

Ethical Considerations

The application of ARTs in conservation biology remains a subject of debate; however, these approaches offer considerable potential for mitigating genetic erosion, reduce extinction risks and preserve genetic diversity of livestock and raises important ethical issues related to animal welfare, genetic integrity, and societal acceptance. Procedures like oocyte retrieval, embryo transfer, and SCNT may cause stress or health risks to animals, while cloned embryos can show developmental or epigenetic abnormalities. Over-reliance on biotechnological methods may narrow genetic diversity and shift resources away from habitat-based conservation. ARTs implementation must be guided by rigorous ethical standards, with careful attention to animal welfare, clearly defined and achievable conservation targets, government policies and conservation programs and the integration of stakeholder participation (Biasetti *et al.,* 2025; Campbell, 2021). Ultimately, the role of ARTs in conservation underscores the intersection between scientific innovation and human responsibility in determining the future of biodiversity. In summary, assisted reproductive technologies can complement conventional conservation strategies by reducing extinction risks and preserving genetic diversity in vulreneable populations. Their success, however, depends on ethically responsible application, realistic objectives, and active stakeholder engagement.

CONCLUSION AND FUTURE PROSPECTIVES

Biobanking and ARTs have revolutionized the conservation efforts of livestock species. Among these, semen and oocyte banking preserve the haploid genetic material from individual animals, while embryo banking through IVEP (including OPU-IVF and ICSI) allows the storage of complete parental genomes, enabling the propagation of endangered animals. Somatic cell banking combined with SCNT and interspecies SCNT provides a valuable tool for supporting conservation

Table 1: Comparative table on the major *ex-situ* conservation tools for livestock species

Tool	What it conserves	Key strengths	Limitations	Typical use
Sperm cryopreservation	Male germplasm	Low cost, well established, scalable	No maternal genome preserved; needs females for recovery	Most common, first step in gene bank
Oocyte/ovarian tissue cryopreservation	Female germplasm	Preserves maternal side; complements semen banks	Technically demanding, recovery complex	Rare or very small populations
Embryo cryopreservation	Both Male + female germplasm	Captures full genetic diversity, including maternal lines	Expensive collection, needs embryo transfer for use	For critical breeds with few females
Somatic cell banking (fibroblasts, tissue culture)	Male and female germplasm in form of somatic cells	Backup for cloning, genomics	Not directly usable without cloning	Last resort safeguard

of critically endangered or extinct species. Together, these technologies offer a comprehensive approach to maintain genetic diversity, safeguard rare breeds, and support sustainable livestock production.

Looking forward, continued advancements in culture media, cryopreservation protocols, epigenetic reprogramming, and automation are expected to improve the efficiency and reliability of these techniques. Integrating multiple ARTs such as using somatic cells from cryobanks in combination with IVEP or SCNT, advanced stem cell based approaches can maximize conservation outcomes. Advanced stem cell based approaches, including induced pluripotent stem cells (iPSCs) and in vitro gametogenesis (IVG), provide powerful tools for conservation. These techniques enable the generation of functional oocytes and sperm from somatic or pluripotent cells, offering unprecedented opportunities to preserve genetic diversity and support the recovery of endangered livestock populations. Expansion of species specific protocols, better understanding of reproductive physiology, and development of standardized procedures will further enhance their applicability. Moreover, the establishment of national and global gene banks, coupled with policy support and ethical frameworks, will ensure long term preservation of livestock genetic resources.

In the future, these technologies are likely to play a pivotal role not only in conserving endangered livestock but also in addressing challenges related to climate change, disease resistance, and sustainable production. By combining biobanking with cutting edge ARTs, it is possible to secure the genetic heritage of livestock species for generations to come, providing a bridge between conservation goals and the practical demands of agriculture.

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