Review Article

Reproductive Biotechnologies for Sustainable Livestock Production: Current Status and Future Directions

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Abstract | The global livestock industry faces persistent challenges in reproductive efficiency, including low conception rates and high embryonic mortality, which hinder productivity and economic sustainability. To address these challenges, modern reproductive biotechnologies have emerged as transformative tools for enhancing livestock productivity and genetic improvement. This review explores the latest developments in assisted reproductive technologies (ART), including artificial insemination (AI), oestrus synchronization, *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), semen and embryo sexing, cryopreservation, embryo transfer (ET), cloning, transgenesis, stem cell technology, and nanotechnology. AI has revolutionized breeding programs by enabling the widespread dissemination of superior genetics, while IVEP and ICSI offer solutions for male infertility and the production of genetically elite embryos. Sexing technologies allow for selective breeding, optimizing resource management in dairy and meat production. Cryopreservation techniques, particularly vitrification, ensure the preservation of valuable genetic material, and ET accelerates genetic progress by leveraging elite donors. Cloning and transgenesis provide innovative avenues for conservation, biomedical research, and trait enhancement. Additionally, stem cell technology and nanotechnology present promising applications for regenerative medicine and precision breeding. Despite these advancements, challenges such as high costs, technical limitations, and field applicability hinder widespread adoption, particularly in developing nations. This review highlights the potential of these biotechnologies to revolutionize animal reproduction while emphasizing the need for further research and infrastructure development to maximize their impact on sustainable livestock production.

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Introduction

The livestock sector plays a vital role in global food security, rural livelihoods, and agricultural economies, contributing significantly to income generation and nutritional supply (Herrero *et al.*, 2013). More than 1.3 billion people worldwide depend on livestock for some part of their livelihood, while the sector contributes around one-fourth of agricultural GDP in many countries (FAO, 2022). Productivity plays a vital role in growth, while reproduction is essential for sustaining animal production. Reproductive inefficiency remains a significant source of economic loss in livestock industries worldwide (Davis and White, 2020; Laghari *et al.*, 2025). Despite its importance, livestock production faces major challenges in reproductive efficiency, including prolonged calving intervals, low conception rates, and high embryonic losses. These inefficiencies not only limit productivity but also impose significant economic burdens on farmers and threaten the long-term sustainability of the sector (Hossein-Zadeh, 2013; Diskin and Kenny, 2016).

To overcome these barriers, modern reproductive biotechnologies have emerged as transformative tools for enhancing fertility, accelerating genetic gain, and conserving valuable germplasm. Assisted reproductive technologies (ART) such as artificial insemination (AI), estrus synchronization, and embryo transfer (ET), and *in vitro* fertilization (IVF) have already reshaped conventional breeding programs by enabling the widespread dissemination of elite genetics. More advanced innovations, including intracytoplasmic sperm injection (ICSI), semen and embryo sexing, cryopreservation, cloning, transgenesis, stem cell applications, and nanotechnology, offer new opportunities to address reproductive limitations with precision and efficiency. Together, these technologies not only enhance productivity but also provide solutions for biodiversity conservation, biomedical research, and adaptation to climate challenges (Singh *et al.*, 2020; Verma *et al.*, 2012; Yousuf *et al.*, 2024).

Recent breakthroughs in ART have enabled innovative manipulation of reproductive processes, transforming global animal agriculture. This review aims to provide insights into the latest ART advancements, offering valuable knowledge to enhance livestock reproduction (Verma *et al.*, 2012). This review provides a comprehensive overview of recent developments in ART and their applications in livestock reproduction. It highlights both established and emerging technologies, discusses their advantages and limitations, and examines the economic and infrastructural barriers hindering their wider adoption, particularly in developing regions. Finally, it considers future directions, emphasizing the need for integrated strategies that combine scientific innovation with practical implementation to achieve sustainable improvements in livestock productivity and genetic diversity. Common assisted reproductive technologies are shown is Figure 1.

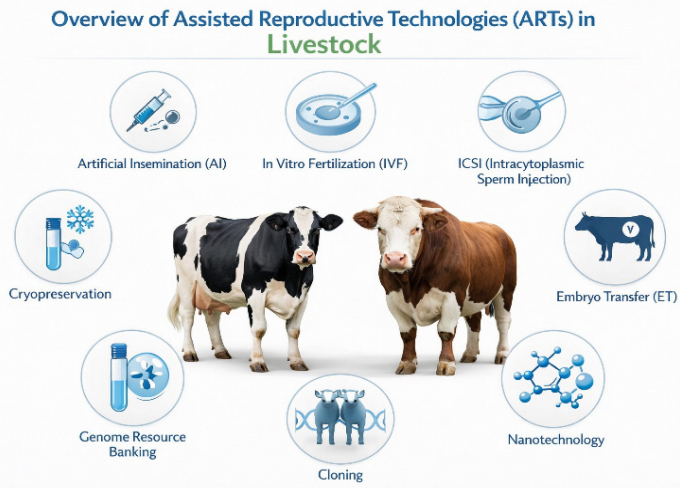

Figure 1: *An overview of assisted reproductive technologies (ART) in livestock.*

Estrus synchronization (ES)

ES is a management technique used to coordinate the reproductive cycles of a group of female animals so that they all enter their fertile period (estrus) within a short, pre-planned timeframe. This advanced practice is especially advantageous for large cattle operations, as it reduces breeding-related errors and lowers overall management expenses (Macmillan, 2010). The core benefits include the ability to schedule breeding and subsequent birthing during the most optimal season, ensuring a suitable environment and ample food for newborn survival. By enabling timely breeding, the technique can also enhance overall herd fertility. Economically, estrus synchronization boosts production efficiency and increases returns by condensing a breeding window that would naturally span about 21 days into a concentrated period of less than five days (Vikrama and Balajai, 2010).

This technique offers significant economic and operational advantages, particularly for large or nomadic herds. It allows for the precise scheduling

of breeding and calving seasons to coincide with optimal environmental conditions and resource availability, thereby improving newborn survival rates. Furthermore, it enables the use of superior genetics through AI across the entire herd at once. Key benefits include the production of a uniform calf crop for future herd replacement, a major reduction in labor and management costs, and a substantial increase in overall production efficiency by streamlining the breeding process (Islam, 2011).

The future of estrous synchronization strategy is moving toward an integrated approach that merges standard control of the corpus luteum's lifespan with direct management of follicular growth. The primary objective is to create a treatment protocol that not only groups estrus more accurately but also precisely determines the timing of ovulation. This would enable a single, predetermined AI for an entire herd, completely eliminating the need for labor-intensive estrus detection (Lamb *et al*., 2010). A promising direction involves combining hormones like Gonadotropin releasing hormone GnRH, Prostaglandin F2α, and progesterone in synchronization programs to effectively manipulate follicular development. The successful design and application of these advanced protocols are fundamentally dependent on a thorough understanding of the hormonal fluctuations and ovarian structures present throughout the different stages of the estrous cycle (Channo *et al*., 2022; Yaniz *et al*., 2004).

Artificial insemination (AI)

Artificial insemination (AI) is a controlled reproductive method in which trained professionals manually deposit sperm taken from a selected male animal into the reproductive tract of a female in order to produce fertilization and pregnancy. This strategy promotes the use of genetically superior sires, increases herd productivity, lowers the risk of venereal disease transmission, and facilitates effective breeding management across large animal populations (Tola and Deresa, 2025). This technology has evolved into a widely adopted practice in commercial dairy farming across both developed and developing nations. Historically, the first successful AI was achieved by Spallanzani (1784) in a dog. The groundwork for modern AI was laid by Ivanoff in Russia in 1899, who later expanded his research to include domestic livestock, dogs, foxes, rabbits, and poultry (Sharma *et al*., 2024). Over time, researchers worldwide adapted this technique for various species. A major breakthrough came with the development of frozen semen, revolutionizing AI by enabling the global distribution of semen (Lonergan, 2018). Initially, AI was employed to enhance indigenous breeds, followed by the promotion of crossbreeding programs. AI offers numerous advantages, including optimal utilization of elite males, rapid dissemination of superior genetics, accelerated genetic selection, cost-effective importation of genetic material via semen rather than live animals, prolonged availability of frozen semen posthumously, and minimized risk of sexually transmitted infections. While AI primarily relies on semen from exotic breeds to boost local livestock productivity, it is also occasionally used with indigenous breeds. Today, AI protocols and methodologies have become highly standardized, ensuring efficiency and reliability in livestock breeding programs.

Currently, AI is practiced on a massive scale worldwide, with over 100 million cattle, 40 million pigs, 3.3 million sheep, and 0.5 million goats inseminated annually (Shukla *et al*., 2023). In India, the production of frozen semen straws reached 44 million in 2008, with 41 million AIs performed at a conception rate of 35% (Annual Report, 2007-2008).

However, in developing nations, the success rate of AI remains disappointingly low, limiting its impact on livestock improvement (Hassoon *et al*., 2025). The primary reasons for this inefficiency include poor management practices and insufficient technical expertise. For AI to deliver optimal results, farmers must have access to better technical support and well-organized facilities. Only then can the full potential of AI be realized in enhancing animal productivity.

In vitro fertilization (IVF)

In vitro Fertilization (IVF) in livestock is an assisted reproductive method in which mature oocytes are extracted from a female animal and fertilized with sperm outside of the body under controlled laboratory settings. The resultant embryos are incubated for a set period of time before being transferred to synchronized recipient females in order to produce pregnancy (Scheuerer, 2009). The commercial viability of mass-producing genetically superior embryos through *in vitro* fertilization has become increasingly evident. Recent decades have witnessed remarkable advancements in *in vitro* embryo

production (IVEP) technologies for livestock species. This progress has accelerated significantly in the last ten years, particularly through the development and optimization of precisely defined and semi-defined culture media tailored for different animal species (Souza-Fabjan *et al*., 2023).

IVEP technologies serve dual purposes: generating genetically superior livestock and supplying high-quality embryos for advanced biotechnological applications including embryo sexing, cloning, nuclear transfer, and transgenesis. These techniques also enable detailed study of embryonic development, facilitating research into gene expression patterns, epigenetic modifications, and cytogenetic abnormalities (Galli and Lazzari, 2008). Bovine embryos are particularly valuable as model organisms due to their developmental similarities with human embryos (Niemann and Wrenzycki, 2000). Despite continuous optimization efforts, bovine IVEP efficiency remains suboptimal, with only 30-40% of matured oocytes developing to blastocyst stage following *in vitro* fertilization and culture (Sirad *et al*., 2006). Initial implementation in cattle and buffaloes was constrained by oocyte recovery challenges, but the advent of minimally invasive ultrasound-guided transvaginal oocyte retrieval (TVOR) and oocyte pickup (OPU) techniques has significantly improved outcomes. The OPU-IVEP system now enables commercial-scale embryo production in multiple countries, with repeated oocyte collections yielding substantially more embryos than conventional embryo transfer methods (Galli and Lazzari, 2008). This approach is particularly valuable for preserving genetic material from endangered or economically valuable livestock species. However, widespread IVEP adoption faces two major limitations: high operational costs and suboptimal efficiency under field conditions, which currently restrict its practical applications.

Time-Lapse Imaging (TLI) allows for continuous monitoring of embryonic development without interrupting the culture conditions. This technology allows (TLI) integrates advanced optical systems with traditional embryo culture methods. By employing time-lapse photography, it captures sequential images of embryo development at regular intervals, offering a continuous and objective view of the dynamic changes during growth. Unlike conventional culture techniques, TLI reduces the risk of missing critical stages, such as the appearance of two pronuclei, thereby improving the utilization rate of eggs and embryos (Chen *et al*., 2019; Leung et al., 2011). Additionally, it maintains a stable environment for embryo development while providing traceable data. This technology allows for precise observation of early embryonic development and accurate recording of developmental time parameters. As a result, it enhances the ability to predict embryo viability, facilitating the selection of a single high-quality embryo for transfer. Ultimately, this contributes to higher clinical pregnancy and live birth rates (Wang *et al*., 2023). Conventional morphological assessment of embryos largely depends on the expertise and subjective judgment of embryologists. While embryos with high morphological scores are more likely to develop into high-quality blastocysts, studies show that even low-scoring embryos have a 25% chance of reaching the same developmental potential and implantation success as their high-scoring counterparts (Zhang *et al*., 2011; Li *et al*., 2019; Stone *et al*., 2014; Lundin and Park, 2020).

Intra cytoplasmic sperm injection (ICSI)

Advancement of ICSI has revolutionized reproductive biotechnology by providing an effective solution for male infertility of various etiologies (Xie *et al*., 2024). This breakthrough has renewed scientific interest in its potential applications for livestock reproduction. Beyond its clinical value in overcoming fertility challenges, ICSI serves two additional critical functions: (1) as a powerful tool for transgenic animal production, and (2) as a research platform for investigating fundamental fertilization.

Since the pioneering success of ICSI in hamsters, this technique has enabled the production of live offspring in diverse species, including cattle, sheep rabbits, humans, horses, mice and pigs (Morishita *et al*., 2021). ICSI involves the microinjection of a single spermatozoon or sperm head directly into the ooplasm, bypassing natural fertilization barriers. Beyond its clinical utility, ICSI serves as a valuable tool for transgenic animal production via sperm-mediated gene transfer. The primary application of ICSI is the treatment of severe male infertility caused by abnormalities in ejaculated, epididymal, or testicular spermatozoa. Success rates vary across species: Cattle exhibit 70–80% fertilization (Fuentes *et al*., 2022), pigs 77% (Coy and Romar, 2022), and small ruminants 48–63% (Briski and Salamone, 2022).

In horses, where conventional *in vitro* fertilization (IVF) is technically challenging and inefficient, ICSI has emerged as the preferred method (Colleoni *et al*., 2007). Despite higher fertilization rates compared to IVF, ICSI often yields suboptimal pregnancy rates (<20%) and shows no significant improvement in clinical pregnancy outcomes. This disparity highlights the need for further refinement of the technique to enhance embryonic viability and post-transfer survival.

Sexing of semen and embryos

Sexing of sperm and embryos in livestock refers to reproductive technology used to predict the gender of progeny before birth. Semen sexing is the process of separating X- and Y-chromosome-bearing sperm cells, typically using flow cytometry, such that insemination results in a higher possibility of creating female or male progeny, depending on production goals. While embryo sexing is the process of determining an embryo's sex at an early developmental stage using molecular or cytogenetic techniques, only embryos of the desired sex are picked for transfer (Chen *et al*., 2025; Kinga and Piotr, 2026). The ability to predetermine offspring sex offers significant advantages in livestock breeding, enabling the selective production of males or females with superior genetics to enhance future generations (Plummer and Beckett, 2006). Sexed embryos used in embryo transfer (ET) programs allow for more efficient resource management, particularly in dairy production, where maximizing heifer calf output is economically beneficial. Sexual differentiation in embryos is governed by genetic markers on the Y chromosome. Several commercially available techniques for embryo sexing include: (1) Chromosomal analysis of demi-embryos, (2) Immunological detection of H-Y antigen, (3) Y-chromosome-specific DNA probes, (4) Fluorescence in situ hybridization (FISH), (5) Loop-mediated isothermal amplification (LAMP), a rapid molecular method for bovine pre-implantation embryos (Zoheir and Allam, 2010). Alternatively, sperm sex-sorting via flow cytometry provides a pre-fertilization approach. This technique involves staining sperm DNA, differentiating X- and Y-chromosome-bearing sperm using a laser, and sorting them for artificial insemination (Garner, 2006). By selectively using X- or Y-chromosome-bearing sperm, this enables the production of embryos of either female or male sex as required. Interestingly, Y chromosome specific DNA sequences in cattle are evolutionarily conserved and shared among buffalo, Indian zebu, and taurine breeds (Alves *et al*., 2010). This genetic conservation makes it possible to use bovine-specific primers for precise sex identification in buffalo and zebu cattle embryos. Advances in embryo biopsy techniques (Lopatarova *et al*., 2008) now enable the non-destructive removal of a single cell from early-stage embryos for genetic sexing using DNA probes. The high post-biopsy survival and conception rates confirm the minimal embryonic damage associated with this procedure. Furthermore, semen sexing via fluorescence-activated cell sorting (FACS) has proven highly effective in producing offspring of predetermined sex across multiple species. Successful applications include: Cattle (Seidel *et al*., 1999), Goats (Parrilla *et al*., 2004), Pigs (Grossfeld *et al*., 2005), Sheep (de Graaf *et al*., 2007). This technology has been validated for both fresh and cryopreserved spermatozoa (Garner *et al*., 2008), demonstrating its versatility in assisted reproductive technologies.

To date, the application of semen and embryo sexing technologies in field conditions has been documented only in China among developing nations. However, active research and development efforts are underway at various institutions across these countries to refine these advanced reproductive techniques. The growing participation of private sector enterprises in offering these services is expected to significantly enhance their availability, particularly in regions where AI infrastructure is already well-established. This commercialization trend may bridge the gap between research innovations and practical implementation in livestock improvement programs.

Genome resource banking

Involves the cryopreservation (freezing) of genetic materials like sperm, eggs, and embryos for future use, providing a way to preserve the genome of individuals, even after their death (Estudillo *et al*., 2021). The success of in IVEP heavily depends on the availability of developmentally competent oocytes, which have a limited fertile lifespan. Cryopreservation of unfertilized oocytes offers a practical solution by creating a readily accessible gamete bank, enabling researchers to conduct experiments at optimal times and preserve valuable genetic material for future use. Over the past few decades, significant advancements have been made in mammalian oocyte and embryo cryopreservation, with live offspring successfully

produced in at least 25 species through the transfer of cryopreserved material (Gajda and Smorąg, 2009). Preservation of oocytes provide several benefits such as: eliminating the need for live animal transport, lowering costs and biosecurity risks, minimizing hazards associated with the movement of biological material, acting as an insurance policy against catastrophic losses and supporting conservation efforts for endangered species. The primary obstacles in germplasm cryopreservation include mechanical and osmotic damage during processing. The introduction of cryoprotectants (CPs) such as glycerol revolutionized the field, improving post-thaw viability.

Vitrification, pioneered by Rall and Fahy (1985), offers a faster, simpler, and more cost-effective approach than traditional slow freezing. Studies demonstrate its superior efficacy for chilling-sensitive materials (Fahy and Wowk, 2015). Despite variable success rates, vitrification has been applied to oocytes in multiple species, including: Bovine, Swine, Equine, and Buffalo (Hurt *et al*., 2000; Hochi *et al*., 2000; Sharma and Loganathasamy, 2007; Huang and Holtz, 2002). Various vitrification techniques such as Electron Microscopy (EM) grid, Open Pulled Straw (OPS), closed pulled straw, and nylon mesh have yielded comparable outcomes, highlighting the versatility of this method.

Recent innovations in vitrification devices have significantly improved the efficiency and convenience of oocyte and embryo cryopreservation. Notable developments include: High-security vitrification devices, pipette tip-based systems, fiber plug techniques, vitrification spatulas, Cryo-E, sealed pulled straws, plastic blade methods, vitri-Inga and cryopette (Almodin et al., 2010; Portmann *et al*., 2010; Muthukumar *et al*., 2008), Rapid-i (Camus *et al*., 2006; Larman and Gardner, 2010; Sun *et al*., 2008; Petyim *et al*., 2009; Tsang and Chow, 2009; Yavin *et al*., 2009; Sugiyama *et al*., 2010). These advancements have enhanced post-thaw survival rates and operational efficiency in assisted reproductive technologies. Embryo freezing is now a well-established commercial practice aimed at preserving embryo viability for subsequent normal development. This technique is particularly critical in ET programs, as embryo viability begins to decline after 12 hours in holding media. Cryopreservation allows temporary storage until optimal transfer conditions are met. For effective genetic management, both male and female embryos should be preserved to maintain: Balanced sex representation, Broad genetic diversity, Conservation of endangered breeds. Cryobanking serves as a vital conservation tool, enabling the establishment of founder populations for potential reintroduction programs (Ptak et al., 2002). The remarkable progress in mammalian oocyte and embryo cryopreservation has been largely driven by vitrification techniques. These methods now offer viable alternatives for the routine breeding programs, commercial embryology applications, and genetic resource preservation across domestic species. The continued refinement of vitrification protocols promises to further enhance the efficacy and accessibility of these technologies in animal reproduction and conservation efforts.

Embryo transfer (ET)

ET technology serves as a vital biotechnology for accelerating livestock genetic enhancement while simultaneously harnessing the reproductive potential of both superior males and females. The application of ET and multiple ovulation embryo transfer (MOET) methods (Kidie, 2019; Younus *et al*., 2023) enables rapid genetic progress, expansion of elite herds, faster herd improvement, and preservation of valuable genetic resources. These advanced reproductive techniques have proven particularly effective for multiplying high-quality genetic stock across multiple species, including cattle (Hasler, 2003), buffalo (Baruselli *et al*., 2020), sheep (Falchi *et al*., 2022), goats (Fonseca *et al*., 2022), horses (Ramirez *et al*., 2023), and swine (Garcia-Canovas *et al*., 2024).

Global implementation of ET technology has shown substantial growth, with approximately 539,680 procedures recorded in 2002, predominantly in dairy cattle. Regional distribution revealed 62% of transfers occurred in North America and Europe, followed by 16% in South America and 11% in Asia (Madan, 2005). More recent data from the International Embryo Transfer Society indicates annual transfers reaching 800,000 bovine embryos, along with 25,000 in sheep, 7,000 in goats, 30,000 in pigs, and 12,000 in equines (Hafez, 2015; Paramio and Izquierdo, 2014). Notably, about two-thirds of these transfers utilized *in vivo*-derived embryos, while the remaining third employed *in vitro*-produced embryos, achieving conception rates between 55-70% (Paramio and Izquierdo, 2014). The procedure of In-vitro embryo production and embryo transfer is illustrated in Figure 2.

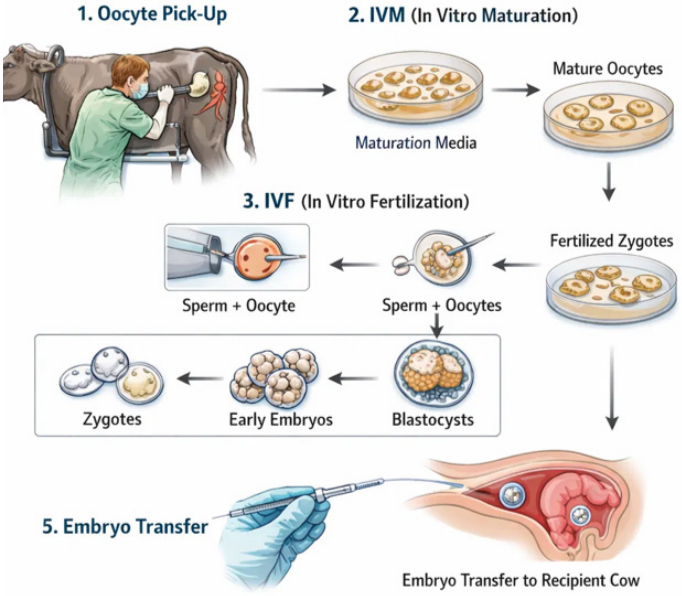

Figure 2: *In vitro embryo production (IVEP) in cattle.*

Embryo genomics

Embryo genomics in animals is the use of modern molecular and genomic technologies to study the genetic makeup of embryos in their early developmental stages. It uses techniques including whole-genome amplification, single nucleotide polymorphism (SNP) genotyping, and genomic sequencing to assess genetic quality, discover inherited diseases and identify desirable production qualities prior to embryo transfer (Johnsson, 2023; Oliveira *et al.*, 2023).

Recent advances in molecular biological techniques are transforming our ability to analyze embryonic development. Innovative approaches including differential display reverse transcription polymerase chain reaction (DDRT-PCR), subtractive cDNA library construction, and quantitative real-time PCR (qRT-PCR) now enable detailed investigation of mRNA expression dynamics during preimplantation stages. These methodologies provide unprecedented opportunities to identify critical gene expression signatures that serve multiple purposes: Embryo quality assessment through molecular marker identification, Developmental normality evaluation for research and clinical applications, Optimization of assisted reproduction technologies. Comprehensive gene expression profiling using both qualitative and quantitative RT-PCR approaches has been conducted in murine and bovine preimplantation embryos (Peynot *et al.*, 2014). These studies have revealed: Approximately 250 functionally significant genes associated with over 32 physiological processes in mouse embryos, between 60-70 developmentally important genes in domestic animal embryos (Kabir *et al.*, 2017). Such molecular characterization establishes a foundation for evidence-based embryo selection and the refinement of reproductive technologies in both human medicine and animal science applications.

Cloning (somatic cell nuclear transfer)

Cloning represents a transformative biotechnology with multiple applications in animal science and conservation. As a reproductive tool, it enables: Genetic preservation of superior livestock specimens, Population management of endangered species, Standardization of research animal genotypes, Therapeutic potential through stem cell production. SCNT has emerged as the predominant cloning technique since the landmark creation of "Dolly" the sheep (Alberio and Wolf, 2021). This method permits selective multiplication of genetically superior animals using various donor cell types, including: Fibroblasts (both fetal and adult origin), Hepatocytes, Granulosa cells, and Lymphocytes (Chehelgerdi *et al.*, 2023). SCNT has been successfully implemented across multiple species: Cattle (Keim *et al.*, 2023), Swine (Nesiyama *et al.*, 2025), Caprine (Skrzyszowska and Samiec, 2021), Equine (Wakchaure and Ganguly, 2016). While embryonic stem cell (ESC) cloning (NTESC) has been achieved in humans and laboratory models, derivation of farm animal ESCs (faESCs) remains challenging (Beyhan *et al.*, 2007). Current research explores alternative cell sources including: Embryonic germ cells (EGCs) and Spermatogonial stem cells (Zhang *et al.*, 2020). This technology continues to evolve, offering promising solutions for genetic conservation, biomedical research, and livestock improvement programs.

Cloning technology offers a revolutionary approach to genetic preservation and reproduction, enabling the large-scale generation of genetically identical animals without relying on traditional breeding methods. This technique is particularly valuable in regions where conventional methods of semen and embryo collection and storage are impractical, as it allows for the conservation of genetic diversity through the replication of diverse animal specimens. Local livestock breeds often possess unique genetic traits such as heat tolerance or disease resistance that are crucial for adaptation to challenging environments. Cloning provides an effective strategy to safeguard these valuable genotypes from extinction.

Beyond conservation, cloning holds significant potential for medical applications, particularly in xenotransplantation. By producing genetically modified pigs with human-compatible tissues, cloning could facilitate the large-scale generation of organs suitable for human transplantation (Duszewska and Reklewski, 2007). This advancement may address critical shortages in donor organs and revolutionize transplant medicine.

According to an Office International des Épizooties (OIE) conducted global survey (MacKenzie, 2005) involving 91 nations (with 60% representation from developing countries), cloning capabilities were reported by only 4% of African respondents and 23% of Asian respondents. This data highlights the uneven global distribution of cloning technology as of 2005. The field has witnessed significant milestones in recent years, including: The successful cloning of camels at Dubai's Camel Reproduction Center Injaz (2009), the world's first female cloned camel and Bin Soughan (2010), a subsequent male cloned camel. Breakthroughs in bovine cloning at India's National Dairy Research Institute (NDRI), Karnal: Development of the innovative "Hand-guided Cloning Technique", Production of Great Advanced Reproductive Innovative Milestone in Animal Biotechnology–I (GARIMA-I) (2009), the first cloned buffalo calf using this method. Subsequent successful clones including "GARIMA-II" (2009) and Shresth (2010), a male buffalo calf. These achievements demonstrate both the geographical expansion and technical refinement of animal cloning technologies since the initial OIE survey. The procedure of cloning technique in goat is shown in Figure 3.

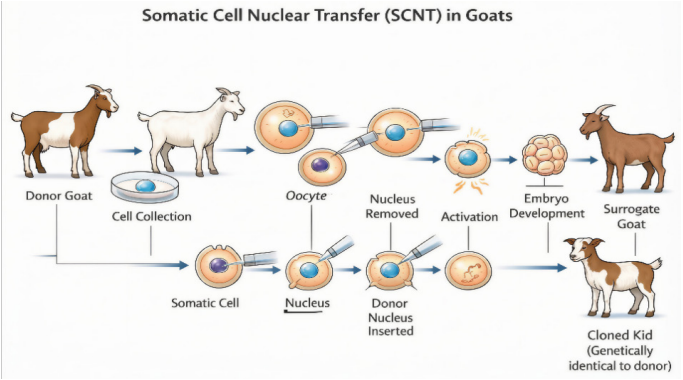

Figure 3: *Process of cloning technique in goat.*

Transgenesis

Transgenesis is a genetic engineering process that involves purposely inserting a foreign or modified gene (transgene) into an animal's genome in order to develop new or improved characteristics. The inserted gene forms a permanent part of the animal's DNA, which can be handed on to future generations (Blanco and Blanco, 2023). The groundbreaking achievement in the 1980s of creating transgenic animals carrying foreign genes marked a transformative milestone in biological and biomedical research. This pioneering work laid the foundation for numerous advances in genetic engineering across multiple species, including transgenic models such as mice (Tasic *et al.*, 2011), pigs (Ivics *et al.*, 2014), sheep (Kalds *et al.*, 2019), goats (Skrzyszowska and Samiec, 2021), and cattle (Keim *et al.*, 2023). Modern transgenic research employs multiple sophisticated biotechnological approaches includes: Pro-nuclear and cytoplasmic microinjection for direct gene insertion, Retroviral vector systems for embryo or embryonic stem cell modification, Lentiviral-mediated sperm gene transfer techniques, RNA interference methods for targeted gene regulation. These diverse methodologies have significantly expanded our capacity to engineer and study genetically modified organisms for both research and applied purposes.

The development of transgenic farm animals has created significant opportunities in both agricultural production and medical research (Robl *et al.*, 2007; Wells, 2010). Within agricultural breeding programs, genetic engineering has enabled the development of livestock with enhanced traits, including: Disease resistance, exemplified by mastitis-resistant cattle (Wall *et al.*, 2005). Improved production characteristics, such as Dairy cattle engineered for enhanced milk composition (elevated κ-casein and β-casein content) (Niemann *et al.*, 2005), Swine with accelerated growth rates and optimized body composition through human growth hormone expression (Niemann *et al.*, 2005), Sheep exhibiting increased wool production via keratin-IGF-I gene integration (Kues and Niemann, 2004). Small ruminants producing therapeutic proteins (antithrombin III and α1-antitrypsin) in milk (Kues and Niemann, 2004). The technique of transgenesis in cattle is shown is Figure 4.

Restoring genetic diversity through gene editing

This technique involves applying advanced molecular tools such as "Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated protein 9" (CRISPR-Cas9), "Transcription Activator-Like Effector Nucleases" (TALENs), and base editing

to reintroduce or modify genes in populations with limited genetic variation, thereby enhancing traits like fertility, resilience, and overall health (Tavakoli *et al.*, 2021; Chen *et al.*, 2024). Recent progress in gene editing has drawn considerable interest in livestock breeding, as it enables precise manipulation of the genome to accelerate genetic improvement (Jenko *et al.*, 2015). This technology can introduce, remove, or modify alleles at specific genomic sites (Gonen *et al.*, 2017), and when applied to zygotes or germ cells, the changes are permanent and heritable. Successful applications in livestock include myostatin (MSTN) gene edits to enhance muscle growth in pigs, cattle, and sheep (Proudfoot *et al.*, 2015); the incorporation of the polled gene in dairy cattle (Tan *et al.*, 2013); and the development of disease resistance in pigs against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and African swine fever (Proudfoot *et al.*, 2015; Lillico *et al.*, 2013, 2016). Although most economically important traits in livestock are quantitative and influenced by numerous small-effect variants, current gene editing efforts have primarily focused on single, major-effect traits (Gonen *et al.*, 2017). More recently, the strategy of promoting alleles by genome editing (PAGE) has demonstrated that editing even a limited number of variants can significantly increase both short- and long-term genetic gain, surpassing the results of conventional selection (Jenko *et al.*, 2015).

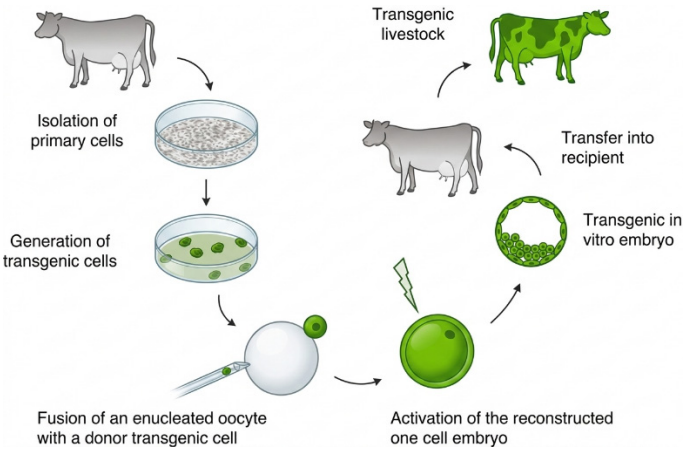

Figure 4: *Production of transgenic livestock.*

Future strategies may include analyzing genetic material from museum specimens to reconstruct the original genetic makeup of species facing extinction (Ansori *et al.*, 2023). These profiles could act as templates for reintroducing lost alleles or haplotypes into present-day populations. By incorporating specific nucleotide changes or entire genes into cultured fibroblasts through gene editing (Doudna and Charpentier, 2014), the modified cells could be developed into induced pluripotent stem cells (iPSCs), which form the basis of *in vitro* gametogenesis. Gametes generated in this way may carry ancestral genetic patterns previously identified in preserved specimens.

Stem cell technology

Stem cell technology in livestock refers to the use of undifferentiated cells with the ability to self-renew and differentiate into specialized cell types for research, breeding, and medicinal applications. Stem cells are extracted, cultivated, and manipulated in animal production systems under controlled laboratory conditions in order to study embryonic development, increase reproductive efficiency, and investigate regenerative treatments (She *et al.*, 2025; Narasimha *et al.*, 2025). Stem cells represent a unique class of undifferentiated cells present in multicellular organisms, characterized by their dual capacity for self-renewal through mitotic division and differentiation into specialized cell types. The main types of stem cells used in scientific research are embryonic, cord blood-derived, adult (somatic), and spermatogonial stem cells. These cell types play a major role in various fields, such as studying developmental biology, advancing transplantation and gene therapies, creating chimeras, supporting drug discovery, and contributing to regenerative medicine (Bajada *et al.*, 2008). In livestock reproduction, embryonic stem cells (ESCs) hold significant potential in two main areas: first, enabling genetic modification via homologous recombination, which can be followed by blastocyst injection for chimera formation and breeding or by somatic cell nuclear transfer; and second, facilitating the creation of large animal models to test tissue-specific differentiation methods and assess cell-based therapies for different organs (Navarro *et al.*, 2024; Narasimha *et al.*, 2025). ESC-mediated gene transfer presents several advantages over alternative transgenic approaches: Enhanced efficiency in generating transgenic animals and capacity for *in vitro* genetic modification (Shakweer *et al.*, 2023), allowing confirmation of stable transgene integration prior to chimera production. Studies have suggested multiple ESC lines from various embryonic sources, including: Blastocyst inner cell mass, individual blastomeres from early-stage embryos and Single-cell stage embryos (Klimanskaya *et al.*, 2006; Hwang *et al.*, 2004).

Spermatogonial stem cell (SSC) transplantation has emerged as a valuable technique for investigating spermatogenesis regulation, with potential applications in managing male fertility. While initially developed in rodent models, this technology has been successfully adapted for use in domestic species including goats, pigs, and cattle (Joerg *et al*., 2003; Honaramooz *et al*., 2003). In bovine reproduction, SSC transplantation offers significant potential for genetic improvement programs. The technique enables dissemination of elite genetics through transplantation of superior bulls' SSCs into recipient males (Herrid *et al*., 2006), Creation of an alternative breeding strategy to AI in regions where AI implementation is challenging (Hill and Dobrinski, 2006). Notably, research has demonstrated successful interspecific and interbreed SSC transplantation in cattle (Herrid *et al*., 2006). Despite these advances, current understanding of stem cell biology remains limited, particularly regarding their full potential in agricultural and reproductive applications. Ongoing research continues to explore and refine these techniques for improved implementation in livestock production systems.

Nanotechnology

Nanotechnology represents a cutting-edge innovation within cellular and molecular biotechnology, holding significant promise to transform the fields of agriculture and livestock production. This technology enables the manipulation of biological materials and fluids at extremely small volumes typically in the nanoliter or picoliter range. Beyond its well established roles in cell biology, genetic engineering, and therapeutic applications, nanotechnology is emerging as a valuable tool in reproductive biotechnology, especially in the breeding of farm animals. Recent advancements such as microfluidic and nanofluidic systems (Yan *et al*., 2023; Alias *et al*., 2021) are being utilized to streamline conventional methods like *in vitro* fertilization (IVF) and embryo culture (Chaplia *et al*., 2024). Notably, microfluidic techniques have proven effective in isolating motile sperm without relying on centrifugation (Yan *et al*., 2023), and they also offer the capability to manipulate oocytes under laboratory conditions (Jahangiri *et al*., 2024). Pioneering work by Glasgow *et al*. (2001) demonstrated that embryos can be successfully manipulated and transported within a microfluidic setup. These systems also provide potential for the sorting of gametes based on specific parameters. Functioning through a network of micro- and nano-scale channels and valves, these platforms are often integrated with computer-based control systems for precise fluid management and data analysis. In addition to reproductive technologies, nanotechnology has made notable contributions to genomics. Its use in genome mapping and sequencing may facilitate the identification of genes linked to economically important traits such as disease resistance and meat quality. By incorporating gene-specific probes onto biochips, breeders can rapidly identify superior animals and eliminate those with undesirable genetic conditions. Moreover, heat detection in livestock can be improved through the subcutaneous implantation of carbon nanotubes (O'Connell *et al*., 2002), which can sense fluctuations in estradiol levels. These sensors may be linked to centralized monitoring systems to automate and optimize breeding strategies.

Economic barriers to the adoption of these biotechnologies in livestock production and strategies to overcome these barriers

Advanced reproductive biotechnologies including AI timed-AI protocols, sex-sorted semen, (ET, and *in-vitro* embryo technologies offer powerful routes to accelerate genetic gain, improve reproductive efficiency, and raise productivity in both dairy and beef systems. However, despite clear biological potential, uptake of these technologies remains uneven, especially among smallholder and resource-constrained producers. The economic gap between potential technical benefits and routine on-farm adoption is a primary bottleneck: when costs (direct and indirect) outweigh perceived or realized returns, farmers rationally avoid investment in technologies that would otherwise improve herd performance (Mikkola *et al*., 2024; Ribeiro *et al*., 2018).

Multiple, interacting economic barriers drive low adoption. First, high upfront and per-service costs particularly for ET, IVF and sex-sorted semen make these options unaffordable for many producers unless clear short-term returns exist; AI is generally cheaper but still carries transaction and service costs that can deter repeated use. Second, limited access to finance and risk-bearing instruments means farmers cannot smooth the investment or absorb failed services (e.g., repeat inseminations, pregnancy losses), raising their effective cost of adoption. Third, small herd size and fragmented production systems reduce the ability of individual farmers to capture economies of scale, so per-animal costs remain high and service providers find it hard to operate profitably. Fourth, weak service

delivery infrastructure (cold chain for semen, reliable technologists, coordinated scheduling) increases operational costs and lowers success rates, which further reduces the expected economic return (Dhraief *et al.*, 2019). Finally, information asymmetries and human capital constraints (limited farmer knowledge, low confidence in new technologies, and inadequate extension/follow-up) depress willingness to pay and reduce observed uptake even where benefits exist. These patterns have been documented across regions and production systems and are major determinants of adoption heterogeneity (Omondi *et al.*, 2017).

Addressing these barriers requires coordinated, economically sensible strategies that reduce costs, share risk, and raise the net benefits perceived by livestock producers. Practical approaches include targeted financial interventions (subsidies for high-cost services, microcredit and input-linked loans, payment-flexibility schemes, and insurance products to cover reproductive failures) to lower the effective price and risk of adoption; organizational models that create scale (community AI hubs, cooperatives, private public service hubs and mobile technician networks) to spread fixed costs and improve service reliability (Omondi *et al.*, 2017). Knowledge and capacity building (demonstration farms, farmer field schools, technician certification, and bundled extension services that integrate fertility management with nutrition and herd health) to raise realized success rates and hence return on investment. Combining these levers can shift adoption from isolated pilots to sustained, scalable programs (Tadele *et al.*, 2025). Modern reproductive biotechnologies, their applications and their challenges are summarized in Table 1.

Table 1: *Modern reproductive biotechnologies in livestock, their applications and their challenges.*

Technology	Applications in livestock	Major limitations / challenges	Citations
Artificial Insemination (AI)	Dissemination of superior genetics, crossbreeding, disease control, cost-effective semen transport	Low success rates in developing nations due to poor management and technical expertise.	Sharma *et al.*, 2024; Lonergan, 2018; Shukla *et al.*, 2023
Estrus Synchronization	Scheduling breeding/calving, uniform calf crop, improved herd fertility	Requires understanding of hormonal cycles and ovarian structures for advanced protocols.	Macmillan, 2010; Islam, 2011; Channo *et al.*, 2022
In vitro Fertilization (IVF) and IVEP	Mass embryo production, embryo sexing, cloning, transgenesis, conservation of endangered breeds	High cost, suboptimal efficiency (30-40% blastocyst rate), limited field applicability.	Galli and Lazzari, 2008; Niemann and Wrenzycki, 2000; Sirard *et al.*, 2006
ICSI	Overcoming male infertility, transgenic animal production	Lower pregnancy rates (<20%) despite high fertilization rates; requires technical refinement.	Morishita *et al.*, 2021; Briski and Salamone, 2022; Fuentes *et al.*, 2022
Sexing of semen/ embryos	Producing offspring of desired sex, dairy herd optimization	Limited field application outside China; requires established AI infrastructure	Garner, 2006; Zoheir and Allam, 2010; Seidel *et al.*, 1999
Cryopreservation (Vitrification)	Long-term storage of gametes/ embryos, conservation of genetic resources	Risk of mechanical/osmotic damage during processing.	Rall and Fahy, 1985; Gajda and Smorąg, 2009; Estudillo *et al.*, 2021
Embryo Transfer (ET)	Rapid herd improvement, multiplication of elite genetics	Requires significant infrastructure and technical skill.	Hasler, 2003; Baruselli *et al.*, 2020; Paramio and Izquierdo, 2014
Cloning (SCNT)	Conservation of endangered species, genetic preservation, biomedical research	Technologically complex; uneven global distribution (low adoption in developing countries).	Alberio and Wolf, 2021; Keim *et al.*, 2023; Nesiyama *et al.*, 2025
Transgenesis	Disease resistance, improved milk/ meat production, pharmaceutical proteins	Public acceptance, regulatory hurdles, ethical considerations.	Niemann *et al.*, 2005; Kues and Niemann, 2004
Gene Editing (CRISPR, TALENs)	Enhancing growth, fertility, disease resistance, restoring genetic diversity	High costs, technical challenges like off-target effects, and regulatory and public acceptance issues	Jenko *et al.*, 2015; Gonen *et al.*, 2017; Chen *et al.*, 2024
Stem Cell Technology	Genetic modification, regenerative medicine, fertility research	Limited understanding of stem cell biology in livestock applications.	Bajada *et al.*, 2008; Navarro *et al.*, 2024; Herrid *et al.*, 2006
Nanotechnology	Sperm selection, oocyte manipulation, biosensors for estrus detection	Emerging technology; not yet widely integrated into standard practice	Alias *et al.*, 2021; Yan *et al.*, 2023; O'Connell *et al.*, 2002

In sum, the economic barriers to advanced reproductive biotechnology uptake are multifaceted cost, finance, scale, infrastructure, and information and they interact to undermine farmer incentives. Overcoming them requires integrated policy, financial, and delivery solutions that reduce costs and risks, build farmer and provider capacity, and create institutional arrangements that capture economies of scale and align incentives across the value chain. When designed with local production realities in mind, such strategies can translate technological promise into measurable productivity and livelihood gains (Masse *et al*., 2020; Ribeiro *et al*., 2018; Dhraief *et al*., 2019).

Conclusion

The rapid evolution of ART has initiated in a new era of possibilities for enhancing livestock productivity, genetic improvement, and conservation. From AI and in IVEP to advanced techniques like cloning, transgenesis, and nanotechnology, these innovations hold immense potential to address longstanding challenges in animal reproduction. While AI remains a cornerstone of genetic dissemination, newer technologies such as embryo sexing, cryopreservation, and somatic cell nuclear transfer offer precision and efficiency in breeding programs. However, the widespread adoption of these biotechnologies is hindered by economic constraints, technical limitations, and infrastructural gaps, particularly in developing regions.

To fully realize the benefits of ART, concerted efforts are needed to optimize protocols, reduce costs, and improve accessibility through training and infrastructure development. Future research should focus on refining techniques like stem cell applications and nanotechnology to unlock their untapped potential in livestock breeding and biomedical applications. By bridging the gap between scientific innovation and practical implementation, these technologies can revolutionize sustainable animal production, ensuring food security and genetic diversity for future generations. The integration of ART into global livestock systems represents not just a scientific advancement, but a critical step toward resilient and efficient agricultural practices.

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Novelty Statement

This review provides a comprehensive and up-to-date synthesis of both conventional and emerging reproductive biotechnologies used in livestock production, ranging from artificial insemination and embryo transfer to gene editing, stem cell technology, and nanotechnology. The article uniquely highlights the role of advanced biotechnologies in genetic improvement, biodiversity conservation, and climate-resilient animal agriculture. Furthermore, it critically examines the economic and infrastructural barriers limiting adoption, particularly in developing countries. This integrated perspective offers valuable insights for researchers, veterinarians, policymakers, and livestock producers seeking sustainable solutions for improving animal reproductive performance and productivity.

Author's Contribution

Sajad Ali Laghari Conceptualized the study, searched the literature and wrote an intial draft. Fazul U Rahman Soomro, Baby yasmeen and Muhammad Rashid Shar equally contributed to write and revised a final draft. Qudratullah Kalwar Reviewed and edited the manuscript.
All authors read and approved the final manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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