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Emerging Assisted Reproductive Technologies in Bovines Driving Genetic Gain and Fertility Optimization

Uttam Kumar Sahu, Brijesh Kumar, M. H. Khan, Mayank Singh, Vishnu Vadera, Amit Kumar, Pradeep Chandra
Division of Animal Reproduction, ICAR–Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India
* Corresponding author: Dr. Uttam Kumar Sahu (uttam33000@gmail.com)
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Abstract

Reproductive efficiency is fundamental to genetic progress and sustainability in bovine production systems. Emerging reproductive technologies have progressively transformed cattle breeding from natural service to precision-guided biotechnology. First-generation advances such as artificial insemination and semen cryopreservation enabled global genetic dissemination, followed by multiple ovulation and embryo transfer for maternal genetic multiplication. The development of ovum pick up–in vitro fertilization systems, cloning, and nuclear reprogramming expanded embryonic control. Genomic selection, SNP-based prediction, and CRISPR-mediated gene editing now allow DNA-level intervention, while digital precision monitoring integrates physiological and molecular data. Collectively, these innovations are redefining herd fertility through integrated, predictive reproductive engineering.

Keywords: Assisted reproductive technologies, Gene editing, Genomic selection, Ovum pick up–in vitro fertilization, Precision reproductive management

INTRODUCTION

Reproductive efficiency is central to productivity, genetic progress, and sustainability in bovine production systems. Fertility influences calving interval, replacement dynamics, and lifetime performance, directly affecting economic viability in both dairy and beef sectors (Lucy, 2001; Walsh et al., 2011). However, intensive genetic selection for production traits and associated metabolic stress have contributed to declining fertility in high-producing cattle populations (Royal et al., 2000; Lucy, 2007). These challenges have shifted bovine reproduction from a largely physiological event to a strategically managed, biotechnology-driven system. Over the past century, reproductive technologies in cattle have evolved through successive generational advances. From semen cryopreservation and artificial insemination to

ovum pick up, genomic prediction, and gene editing, each technological phase has expanded control over gametes, embryos, and ultimately the genome. Contemporary research further emphasizes the importance of physiological optimization particularly uterine hemodynamics and follicular-luteal dynamics in determining oocyte developmental competence and fertility outcomes (Sahu, 2025; Sahu et al., 2026). Moreover, morpho-molecular characterization of OPU-derived oocytes has highlighted the critical role of donor management and breed-specific responses in enhancing embryo developmental potential (Donadkar et al., 2024). These findings reinforce that emerging reproductive technologies must integrate biotechnology with reproductive physiology to maximize success.

FOUNDATIONS AND FIRST-GENERATION TECHNOLOGIES

The first generation of reproductive technologies established foundational control over fertilization and genetic dissemination. Artificial insemination (AI) enabled rapid multiplication of superior sire genetics while reducing disease transmission and logistical limitations (Foote, 2002). The parallel development of semen evaluation techniques and cryopreservation protocols allowed long-term storage of viable spermatozoa in liquid nitrogen, marking the beginning of organized germplasm preservation. Advances in cryobiology improved post-thaw sperm survival through optimized extender composition, cryoprotectants, and controlled freezing rates. Early estrus detection and basic hormonal synchronization strategies further improved breeding precision by aligning ovulation timing with insemination, enhancing conception efficiency. These first-generation technologies formed the structural platform for subsequent reproductive interventions. While primarily focused on gamete preservation and timing control, they laid the groundwork for later embryo-based, molecular, and genomic innovations that now define modern bovine reproductive biotechnology.

SECOND GENERATION ASSISTED REPRODUCTIVE TECHNOLOGIES

The second generation of bovine reproductive biotechnology shifted the focus from sperm dissemination to controlled multiplication of superior female genetics. Multiple ovulation and embryo transfer (MOET) became the defining advancement of this phase. By administering exogenous follicle-stimulating hormone, multiple dominant follicles could be induced within a single estrous cycle, allowing recovery of several embryos from a high-genetic-merit donor (Mapletoft and Hasler, 2005). This approach dramatically increased the reproductive output of elite females beyond the natural limitation of one calf per year. Recovered embryos were evaluated morphologically, graded for quality, and transferred into synchronized recipient cows to complete gestation. The refinement of embryo

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cryopreservation and vitrification techniques further enabled storage, transport, and international exchange of bovine embryos while maintaining acceptable post-thaw viability. These developments accelerated genetic gain while strengthening biosecurity by minimizing live animal movement. Beyond commercial multiplication, MOET also contributed to germplasm conservation strategies, particularly for indigenous breeds and genetically valuable lines. Embryo banking provided a safeguard against genetic erosion and supported structured breeding improvement programs. Second generation technologies thus expanded reproductive control from fertilization management to embryonic multiplication. If first generation tools allowed control of sperm and timing, MOET allowed strategic amplification of maternal genetics setting the stage for laboratory-based embryo production systems in the next technological phase.

THIRD GENERATION IN VITRO AND ADVANCED EMBRYO TECHNOLOGIES

The third generation of reproductive biotechnology moved embryo production from the uterus into the laboratory. Ovum pick up (OPU), performed through transvaginal ultrasound-guided follicular aspiration, enabled repeated retrieval of oocytes from elite donors independent of the stage of the estrous cycle (Pieterse et al., 1988). This innovation broke the constraint of superovulation dependency and allowed more frequent and strategic oocyte collection. Retrieved oocytes undergo in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) under controlled laboratory conditions, resulting in embryo development outside the maternal reproductive tract (Galli et al., 2003). This OPU-IVF pipeline significantly increased embryo yield from genetically superior females and became central to commercial embryo production programs (Fig. 1). Importantly, developmental competence of OPU-derived oocytes is influenced by donor physiology, follicular environment, and uterine hemodynamics. Spectral Doppler assessment of uterine blood flow has demonstrated associations between vascular dynamics and reproductive potential (Sahu et al., 2026). Additionally, detailed morpho-molecular evaluation of oocytes retrieved from FSH-stimulated Tharparkar donors has provided insight into cytoplasmic maturity, gene expression patterns, and embryo developmental outcomes (Donadkar et al., 2024). These findings underscore that laboratory success remains biologically anchored to in vivo follicular and vascular health (Sahu, 2025). Advanced embryo micromanipulation techniques, including preimplantation embryo assessment, sexed embryo production, and improved cryopreservation systems, further refined this generation. Third generation technologies thus represent a transition from reproductive assistance to reproductive engineering where gamete interaction, fertilization, and early embryogenesis are precisely controlled outside the animal.

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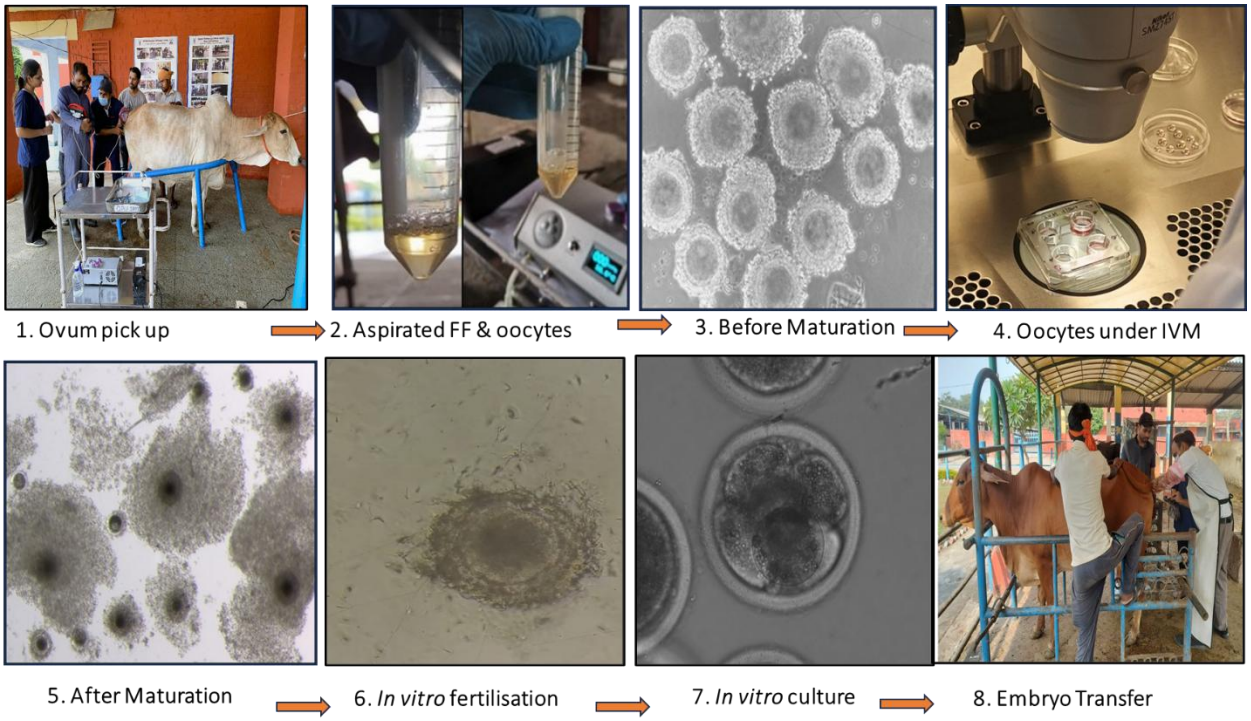

Fig. 1 *In vitro* embryo production

CLONING AND NUCLEAR TECHNOLOGIES

Cloning in bovines represents a transition from managing reproduction to replicating genetic identity. Broadly, two types of cloning approaches have been applied: embryo splitting, often referred to as "handmade" or artificial twinning, and somatic cell nuclear transfer (SCNT), the more advanced nuclear reprogramming technique. Embryo splitting is the simpler form of cloning. In this method, a naturally fertilized early-stage embryo (typically at the 2–8 cell stage) is mechanically divided into two or more parts, each capable of developing into a genetically identical individual. Because the cells at this stage are totipotent, each blastomere retains the potential to form a complete organism. This technique essentially mimics the natural formation of identical twins. It does not alter the genome and does not involve nuclear reprogramming. Its applications are mainly in duplicating high-quality embryos obtained through MOET or IVF programs. Somatic cell nuclear transfer (SCNT), in contrast, is true reproductive cloning. In SCNT, the nucleus of a differentiated somatic cell such as a skin fibroblast from a donor animal is transferred into an enucleated oocyte. The reconstructed oocyte is then activated to initiate embryonic development (Wilmut et al., 1997; Cibelli et al., 1998). Unlike embryo splitting, SCNT does not require fertilization and allows replication of a fully mature elite animal. This method has been used to clone high-genetic-merit dairy cows, elite beef sires, and animals with exceptional production or breeding values. The major difference between embryo splitting and SCNT lies in biological complexity and genomic resetting. Embryo splitting works with an already fertilized embryo and preserves its

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natural epigenetic programming. SCNT requires complete reprogramming of a differentiated nucleus back to an embryonic state, a process that is biologically demanding and often inefficient. As a result, SCNT is associated with lower success rates, higher embryonic loss, placental abnormalities, and neonatal complications due to incomplete epigenetic remodelling. In essence, embryo splitting duplicates early embryonic potential, whereas SCNT attempts to rewind cellular time. One is mechanical duplication of a developing embryo; the other is molecular reprogramming of genetic identity. Both expanded the boundaries of bovine reproductive biotechnology, but SCNT marked the conceptual leap from copying embryos to copying genomes.

GENOMIC AND MOLECULAR ERA TECHNOLOGIES

If cloning allowed us to copy genomes, the genomic era allowed us to read and predict them. The introduction of dense single nucleotide polymorphism (SNP) panels transformed cattle breeding from phenotype-based selection to DNA-informed decision making (Meuwissen et al., 2001; VanRaden et al., 2009). Thousands of SNP markers distributed across the bovine genome are now used to calculate genomic estimated breeding values (GEBVs), enabling accurate prediction of genetic merit early in life often before an animal reaches sexual maturity. This advancement dramatically reduced generation intervals and accelerated cumulative genetic gain, particularly when integrated with artificial insemination and embryo technologies. Instead of waiting years to evaluate milk yield, fertility, or carcass performance, breeders can now select calves based on genomic potential within months of birth. Marker-assisted selection further refined this approach by targeting specific genomic regions associated with fertility, disease resistance, feed efficiency, and production traits. Beyond DNA sequence variation, functional genomics introduced transcriptomics, proteomics, and metabolomics into reproductive evaluation. These platforms identify molecular signatures linked to oocyte competence, uterine receptivity, and early embryonic survival. Research exploring uterine vascular dynamics and follicular-luteal characteristics highlights how genomic potential must operate within a physiologically supportive environment (Sahu, 2025; Sahu et al., 2026). The integration of genomic selection with OPU-IVF pipelines allows preselection of elite donors based on SNP profiles before embryo production begins, increasing efficiency and reducing resource waste. In this generation, reproduction is no longer guided solely by observable traits but by probabilistic genomic architecture. Selection shifts from what the animal expresses to what its DNA predicts.

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GENE EDITING AND PRECISION GENETIC ENGINEERING

If genomic selection allowed us to predict genetic merit, gene editing introduced the possibility of directly modifying it. Precision genome engineering tools such as CRISPR-Cas systems enable targeted alteration of specific DNA sequences within the bovine genome. Unlike traditional breeding, which reshuffles existing variation, gene editing can introduce, delete, or modify defined genetic regions with high specificity (Proudfoot et al., 2015; Carlson et al., 2016). CRISPR-Cas functions as a molecular scissors guided by RNA sequences that recognize complementary DNA targets. Once the DNA is cut, natural cellular repair mechanisms introduce desired modifications. In cattle, gene editing has been explored for traits such as disease resistance, improved thermotolerance, and polled (hornless) phenotypes without altering other production characteristics (Fig. 2). Earlier genome editing platforms such as TALENs and zinc finger nucleases (ZFNs) laid the groundwork, but CRISPR improved efficiency, precision, and scalability. In reproductive biotechnology, gene editing is often combined with embryo-based technologies. Edited embryos are generated in vitro and transferred into recipients, linking molecular engineering directly with OPU-IVF systems. This integration allows modification at the embryonic stage before implantation, embedding desired traits into the germline. However, technical precision does not eliminate biological complexity. Off-target effects, mosaicism, regulatory oversight, and ethical considerations remain significant challenges. Moreover, long-term impacts on animal health and genetic diversity require careful evaluation. Gene editing represents a conceptual shift from selecting superior genetics to constructing them. Where earlier generations preserved, multiplied, or predicted genetic merit, this phase introduces intentional genomic redesign transforming reproduction from management of inheritance to potential programming of it.

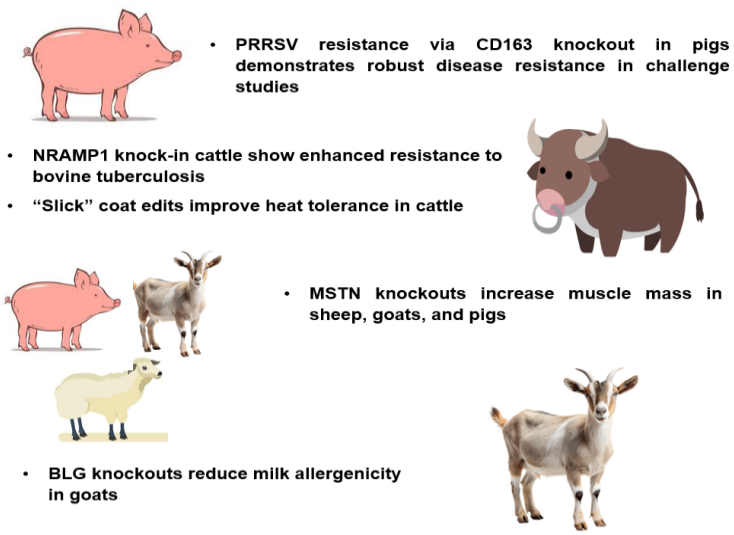

Fig. 2 Major gene edits in goats, sheep, pig and cattle

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DIGITAL AND PRECISION REPRODUCTIVE MANAGEMENT

As molecular tools refined the genome, digital technologies began refining management decisions in real time. Precision reproductive management integrates sensor-based monitoring, imaging technologies, and data analytics to predict and optimize fertility outcomes before failure occurs. Automated estrus detection systems use accelerometers, pedometers, and activity collars to identify behavioral changes associated with heat. These systems improve timing accuracy for insemination compared to visual observation alone, particularly in large herds where subtle estrus signs are easily missed. Continuous progesterone monitoring through milk or blood biosensors further enhances precision by identifying luteal function, anovulation, or early embryonic loss. Advanced ultrasonography, including Doppler imaging, provides functional assessment of ovarian and uterine blood flow. Spectral Doppler evaluation of the middle uterine artery has demonstrated associations between vascular dynamics and reproductive status, offering insights into uterine receptivity and follicular-luteal competence (Sahu et al., 2026). Such physiological metrics bridge the gap between molecular potential and functional fertility. Artificial intelligence and big data analytics now integrate genomic information, hormonal profiles, milk yield data, and behavioral metrics into predictive fertility models. Algorithms can estimate optimal breeding windows, identify subclinical reproductive disorders, and stratify animals by reproductive risk. In this generation, reproduction becomes a continuously monitored biological system rather than a periodic event. Digital precision management does not replace biotechnology; it synchronizes it. Genomic prediction, embryo production, and gene editing achieve maximum efficiency only when supported by real-time physiological data. The final stage looks ahead toward integrated platforms combining biotechnology, genomics, and digital systems for predictive and climate-resilient reproductive engineering.

FUTURE PERSPECTIVES AND CONCLUSION

Bovine reproductive biotechnology is advancing toward integrated, predictive systems that combine assisted reproductive technologies, genomic selection, gene editing, and digital precision monitoring. The future emphasis will be on climate resilience, improved fertility efficiency, and sustainable genetic progress through data-driven reproductive planning. From artificial insemination and semen cryopreservation to OPU-IVF, cloning, SNP-based genomic selection, and CRISPR-mediated editing, each technological generation has expanded the scope of reproductive control. Collectively, these innovations have transformed bovine fertility into a precision-guided, biotechnology-integrated discipline. The next phase will

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depend on intelligent convergence of physiology, molecular science, and digital analytics to ensure resilient, efficient, and sustainable cattle production.

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