



Advances In Assisted Reproduction In Ruminants

Dr. J. G. Kalaria^{*}, Dr. H. M. Patel¹, Dr. M. M. Islam², Dr. P. H. Agravat³, Dr. J. H. Patel⁴

¹MVSc Scholar, Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

²Associate Professor and Head, Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

³Assistant Professor, Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

⁴Associate Professor and Head, Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat, India

*Corresponding author: kalariajanakg05@gmail.com
doi.org/10.5281/VeterinaryToday.23322426

Abstract

Livestock sector has been showing advancing trends in India's economy with its increasing contribution year-on-year to the Agriculture and Allied Sector Gross Value Added (GVA) in the last 15 years or so which has been validated by reports. This growth rate can further be increased by usage of Assisted Reproductive Technologies (ART) so as to explore the potential of vast indigenous genetic diversity of various farm animals in India. ART have completely changed modern dairy farming. They have transitioned simple breeding methods to advanced reproductive technologies. Three main tools used to improve dairy animals currently are Artificial Insemination (AI), Sex-sorted semen and *In Vitro* Fertilization-*In Vitro* Embryo Production-Embryo Transfer (IVF-IVEP-ET). There are various standard operating procedures for each method to mimic the *in vivo* conditions occurring in animals. Additionally, these methods tend to enhance the quality of results as it would be obtained by using conventional natural mating technique. By using these methods correctly, veterinarians and farmers can get better pregnancy rates, speed up the breeding of superior animals and avoid the monetary losses caused by unwanted male calves.

Keywords: Artificial Insemination, Assisted Reproductive Technologies, Embryo Transfer, *In Vitro* Fertilization, *In Vitro* Embryo Production, Ruminants, Sex-sorted semen

Introduction

Agriculture and allied sector have been a key contributor to Gross Value Added (GVA) in India's economy with a contribution of 18.2% to total GVA in the year 2022-23

(Ministry of Agriculture and Farmers, 2025). Livestock sector contributes around 30% in Agriculture and allied sector GVA for the year 2022-23. Apart from this, livestock has been showing an increase in the percentage share



from around 24% in the year 2014-15 to around 30% in 2022-23 in the agriculture and allied sector GVA (Ministry of Finance, 2024). In the modern dairy sector, maximizing reproductive efficiency and accelerating genetic merit are vital for economic sustainability and herd improvement (Vishwanath & Moreno, 2018; Sjunnesson, 2019). ART have evolved from basic management options into highly sophisticated, physiological interventions that manipulate gametes and the uterine environment to optimize maternal and paternal selection intensities (Hafez & Hafez, 2000; DeJarnette *et al.*, 2011).

An understanding of these reproductive technologies is essential for practicing veterinarians, livestock inspectors and AI workers so as to improve the current scenario of livestock production in India (Kumar, 2018; Jaglan *et al.*, 2025). ART used at field level today are AI with normal semen straw or with sex sorted-semen. The usage of IVF-IVEP-ET technology remains more or less in laboratories having necessary equipment and targeted workforce. Rastriya Gokul Mission has been started by Government of India in 2014 so as to conserve its Indigenous Genetic Resources and also to upgrade its cattle population (Annual Report 2024-25, DAHD), hence information regarding the scientific basis, precise execution protocols, and clinical outcomes of ART becomes important for concerned professionals. (Roberts, 1986; Lopez-Gatius, 2022).

Artificial Insemination

Principle

The special requirements (space, nutrition, exercise, etc.) when keeping male animals on a livestock farm can be removed by using the AI method for reproductive management. AI is frequently used ART in large ruminants compared to small ruminants due to difficulty in detecting estrus in the later ones as a result of large flock size, small body, and seasonality in breeding. Keeping a male animal for breeding purpose in small ruminants would be an advantage. AI further helps in

selection of bulls and manyfold transfer of its desired genes in vast population of female animals as compared to when it's used via natural mating. Department of Animal Husbandry, GOI provides door step facility to livestock owners for carrying out AI in their animals. AI provides the ease of using the bull's semen as and when required as compared to managing the whole animal on a farm.

The success of artificial insemination depends entirely on synchronizing sperm deposition with the physiological timeline of the estrous cycle. A typical bovine estrous cycle averages 21 days, with a standing estrus duration of 12 to 18 hours. Ovulation occurs post-estrus, approximately 10 to 14 hours after the cessation of standing heat (or 24 to 30 hours from the onset of estrus phase of estrus cycle). Cryopreserved and thawed spermatozoa require 6 to 8 hours within the female reproductive tract to undergo capacitation involving the vital biochemical modifications; therefore, insemination should be done before ovulation (Lopez-Gatius, 2022). Commonly used technique for deciding when to do AI is the 'AM-PM Rule' given by Trimberger which says that cows showing standing heat in the morning must be inseminated in the late afternoon of the same day and cows showing standing heat in the afternoon must be inseminated the following morning.

Modern transrectal ultrasonography demonstrates that performing AI 16 to 6 hours before ovulation provides the peak conception rate (CR) (Lopez-Gatius, 2022). Clinical indicators of late estrus include high tone and turgidity of the uterine horns, relaxation of the cervix, and a clear, thick, scanty and copious cervical mucus discharge.

Necessities in AI

Semen straws are readily available in market these days. Semen is preserved in 0.25 mL or 0.50 mL polyvinyl straws stored in liquid nitrogen containers at -196°C. The handling of frozen semen straws is a critical point where procedural errors can lead to sperm mortality. Though some scientists contradict this view.

Koch *et al.*, (2022) used three different thawing temperature-time combinations with no significant difference in result. 38 °C for 11 sec, 38 °C for 35 sec, vaginal or cervical thawing of semen for 30 sec prior to deposition in uterus. In earlier two combinations gun was pre-warmed to 35 °C whereas in later no warming was done. Further, their research says that number of lactations, month of the year and AI during natural heat or in combination with oestrus synchronization protocols could affect the CR. Thus, maintaining the thawing SOP (thawing at 35-37 °C for 30-40 sec and loading straw in pre-heated gun) and also managing cow-side parameters can jointly improve the CR.

Site of Insemination

Scientists have done many researches to determine the best site to deposit semen during performing AI. Most of them reveals that best CR could be obtained when semen is deposited at mid of cervix and uterine body. Depositing semen in either of the horns reduces the chances of conception in cases of ovulation from contralateral side.

Sex-Sorted Semen Technology

Principle

Sex-sorted semen technology addresses the strong economic preference for female calves in the dairy industry (Vishwanath & Moreno, 2018). It works on the principle of flow cytometry/cell sorting (Seidel, 2014). The technology relies entirely on the difference in genomic DNA content between X- and Y-chromosome-bearing spermatozoa. In bovine species, the X-chromosome is significantly larger, containing approximately 3.8% to 4.2% more DNA than the Y-chromosome (Jaglan *et.al.*, 2025).

Sex-sorted semen is used by integrating with AI in place of conventional semen straw. Female calves with accuracy of above 90% could be produced using sex-sorted semen. (Annual Report 2024-25, DAHD). Despite high sexual preference, it exhibits inherent physiological compromises that create a 'fertility gap' compared to conventional semen

(Vishwanath & Moreno, 2018). Two reasons for reduced fertility when using sex-sorted semen are less sperms per semen dose and damage to sperms due to exposure to various physical and chemical stress during sorting process (Seidel, 2014). Chances of success can be increased by careful selection of the female animals having regular breeding records and advancing the site of insemination to deep uterine area of the ipsilateral horn after ultrasonographic examination of dominant pre-ovulatory follicle.

In Vitro Fertilization and Embryo Production Protocols

Principle

AI and sex-sorted semen are used mainly used to rapidly transmit the genetic potential of the proven male animals to the field level. IVF works similarly but is used mostly for transmitting the genetic traits of high yielding female animals. IVF works in three steps as mentioned below:

(a) Ovum Pick-Up (OPU)

It requires the collection of oocytes. High producing females can be used as oocyte donors. Ultrasound-guided OPU can be done under caudal epidural anesthesia. Follicles are visualized by transrectal manipulation of the ovary against the vaginal wall, punctured via the vaginal fornix with a special needle, and aspirated continuously. After pickup, depending on the number of layers of cumulus cells surrounding the oocyte, grading of cells is done. Cumulus-Oocyte Complexes (COCs) were divided into 4 grades based on cumulus and nucleus cells: Grade A/Grade 1 – complete covering of cumulus cells around the oocyte; Grade B/Grade 2 – partial covering of cumulus cells around the oocyte; Grade C/Grade 3 – no cumulus cells covering around the oocyte; and Grade D/Grade 4 - degenerated cumulus cells and oocyte. Grades A/ Grade 1 and Grade B/ Grade 2 are taken as typical, while grades C and D were considered to be abnormal (Khandoker *et al.*, 2001; Khandoker *et al.*, 2005; Khandoker *et al.*, 2012).

(b) IVF and In Vitro Embryo Production

(IVEP)

It further involves three sub-stages:

- *In Vitro* Maturation (IVM) of collected oocytes,
- *In Vitro* Fertilization (IVF) using the matured oocytes and cryopreserved-thawed semen, and
- *In Vitro* Culture (IVC) of zygotes to a necessary growth stage required for implantation *in vivo*.

Different media are used for three different sub-stages. In addition to this, stage specific time-temperature-moisture combinations needs to be maintained. This stage ends with the formation of embryo which can be used for implantation *in vivo* in recipient animals. *In vitro* embryo production (IVEP) bypasses maternal tract limitations, allowing a single elite donor female to produce a high volume of offspring (Sjunnesson, 2019).

(c) Embryo Transfer (ET) in recipient females

Chances of success in ET depends largely on the quality of the embryo being used. Prior to embryo transfer, estrus synchronization must be carried out in recipient animal so that the embryo produced by IVF-IVEP gets suitable environment for implantation and further development. Quality of the embryo directly affects pregnancy rates (Erdem *et al.*, 2020). The grading of embryo can be done as per guidelines of International Embryo Technology Society. Grading is done on the basis Stage of Embryo Development (1–9 point scale) and Embryo Quality (1–4-point scale). Table 1 enlists various factors affecting the success in establishing pregnancy using ET.

Table 1. Factors affecting establishment of

pregnancy using ET (Akram, 2026)

Donor Cow-Related Factors	Lactational status of animal, Parity, Reproductive health, nutrition, BCS, Season, Genetics (donor merit), etc.
Embryo-Related Factors	Embryo stage, Embryo quality, Fresh/frozen embryo, IVF/ <i>in vivo</i> embryo production, Sex-sorted semen, etc.
Recipient Cow-Related Factors	Lactational status of animal, Parity, Reproductive health, nutrition, BCS, Season, Synchronization of estrus and timing of ET, etc.
Technician/Procedural Factors	Experience of technician, clean work, Correct timing, etc.
Environmental and Management Factors	Heat stress on farm, Synchronization efficiency, nutritional management

Conclusion

The integration of ART such as AI, Sex-sorted semen, IVF-IVEP-ET along with livestock production helps in enhancing the production potential of concerned species. These technologies not only help in disseminating superior germplasm at a rapid rate as compared to conventional breeding methods but also helps in thoroughly utilizing the breeding capacity of animals delivering best possible offsprings thereby uplifting the genetic merit. While each technique possesses unique physiological variations, precise handling



requirements, and different costing, their proper execution remains the key in obtaining results. Ultimately, the application of ART provides a sustainable pathway to bridge the gap between genetic potential and farm profitability in modern livestock production.

References

- Akram, S. (2026). Embryo transfer in dairy herds: A comprehensive review of factors affecting pregnancy success from donor to embryo and recipient. *Veterinary Research Communications*, 50(5), 399.
- DeJarnette, J. M., Nebel, R. L., & Marshall, C. E. (2011). Evaluating the commercial utility of sex-sorted semen in dairy heifers and cows. *Journal of Dairy Science*, 94(1), 433–443.
- Erdem, H., Karasahin, T., Alkan, H., Dursun, S., Satilmis, F., & Guler, M. (2020). Effect of embryo quality and developmental stages of pregnancy rate during fresh embryo transfer in beef heifers. *Tropical Animal Health and Production*, 52(5), 2541–2547.
- Hafez, E. S. E., & Hafez, B. (2000). *Reproduction in Farm Animals* (7th ed.). Lippincott Williams & Wilkins.
- Jaglan, K., Dhaka, S. S., Dahiya, S. P., Ratwan, P., Patil, C. S., Ghanghas, A., & Sheoran, N. K. (2025). Exploring the genetic dimensions of sex-sorted semen: A review. *Bhartiya Krishi Anusandhan Patrika*, 40(2), 129–137.
- Khandoker, M. A. M. Y., Ali, M. R., Islam, M. R., Rahman, M. G. M., & Talukder, M. A. S. (2005). *In vitro* culture of mouse embryos. *Progressive Agriculture*, 16, 121–128.
- Khandoker, M. A. M. Y., Jahan, N., Asad, L., Hoque, S. A. M., Ahmed, S., & Faruque, M. O. (2011). Qualitative and quantitative analysis of buffalo ovaries, follicles and oocytes in view of *in vitro* production of embryos. *Bangladesh Journal of Animal Science*, 40(1–2), 23–27.
- Koch, J., Weber, L. P., Heppelmann, M., Freise, F., Klingelmann, M., & Bachmann, L. (2022). Effect of different thawing methods for frozen bull semen and additional factors on the conception rate of dairy cows in artificial insemination. *Animals*, 12(18), 2330.
- Kumar, P. (2018). *Applied Veterinary Gynecology and Andrology* (2nd ed.). Kalyani Publishers.
- Lopez-Gatius, F. (2022). Revisiting the timing of insemination at spontaneous estrus in dairy cattle. *Animals*, 12(24), 3565.
- Ministry of Agriculture and Farmers Welfare. (2025). *Annual report 2024–25*. Government of India, Department of Agriculture & Farmers Welfare. https://www.agriwelfare.gov.in/Document/s/AR_Eng_2024_25.pdf
- Ministry of Finance. (2024, July). *Allied Sectors of Indian Agriculture have Emerged as Promising Sources for Improving Farm Incomes: Economic Survey* (Press Release). Press Information Bureau, Government of India. <https://www.pib.gov.in/PressReleaseIframePage.aspx?PRID=2034940&reg=3&lang=2>
- Roberts, S. J. (1986). *Veterinary Obstetrics and Genital Diseases (Theriogenology)* (3rd ed.). David & Charles Inc.
- Seidel, G. E., Jr. (2014). Update on sexed semen technology in cattle. *Animal*, 8(1), 160–164.
- Sjunnesson, Y. (2020). *In vitro* fertilization in domestic mammals-a brief overview. *Upsala Journal of Medical Sciences*, 125(2), 68–76.
- Vishwanath, R., & Moreno, J. F. (2018). Review: Semen sexing – current state of the art with emphasis on bovine species. *Animal*, 12(1), 85–96.
- Yahia Khandoker, M. A. M., Imai, K., Takahashi, T., & Hashizume, K. (2001). Role of gelatinase on follicular atresia in the bovine ovary. *Biology of Reproduction*, 65(3), 726–732.