**Assisted Reproductive Technology and its application in livestock**

**Ajoy Ghosh1** , **Baishakhi Saha2 and Arindam Bhowmik3**

Department of Animal Reproduction Gynaecology and Obstetrics

College of Veterinary Sciences and Animal Husbandry, Selesih, Aizawl

Central Agricultural University (CAU),Imphal

**Introduction**

The technical description of assisted reproduction technology (ART) is reproductive technology used to improve the genetic material of livestock.

For animals, it generally includes four successive generations of assisted reproductive technologies (ART). The commercial application of the first three generations of ARTs, which involves artificial insemination (AI), multiple ovulation and embryo transfer (MOET), in vitro fertilization (IVF) procedures, and gamete and embryo sexing, is successful. On the other hand, the fourth generation of ARTs, which includes nuclear transfer (NT) of somatic or embryonic cells, genetic marker-assisted selection (MAS), stem cell biology, and transgenic animals, tends to be less successful.Even though their commercial uses have been relatively small, the third and fourth generations of assisted reproductive technologies (ART) provide the potential for improving the breeding rate of superior animals.

The fourth generation of assisted reproductive technologies is increasingly being implemented. The categories that follow are the fourth-generation ARTs: -

1. **Genetic marker-assisted selection =** Genetic marker-assisted selection has been implemented in association with phenotype in recent years. Previously, selection for breeding in livestock was carried out on the basis of superior phenotypes.

The widespread availability of information on the animal genomes can be used to improve breeding programs through the improvement of desired features. Quantitative trait loci (QTL) are chromosomal regions or loci that are responsible for many traits, each of which might impact the trait's variability [1].

Genetic marker-assisted selection (MAS) is the method by which genetic markers have been used to identify the target loci that specifically affect the single desired trait gene and also quantitative trait loci (QTL) [10]. The size of the QTL determines the genetic gain in MAS [2]. By way of example, there are commercial markers that have been linked to cow tenderness [1].

1. **Nuclear transfer cloning (NT) =** Nuclear transfer cloning is a form of cloning in which the nucleus of a somatic cell has been introduced into an enucleated metaphase-II oocyte, which leads to the generation of an animal new who is genetically identical to the somatic cell donor.

While working at the Roslin Institute in Roslin, Scotland, in the 1990s, Ian Wilmut, Jim McWhir, and Keith Campbell conducted studies. Many sheep were primarily utilized in the experiment, including those born in July 1996. One of these sheep, Dolly, was born on July 5 of that year's experiment. The first sheep to be commercially cloned, Dolly, was developed from the nuclei of fully differentiated adult cells as opposed to those of early embryonic cells [4].

Low efficiency and a high chances of developing defects are complications with somatic cell cloning [12–18].Currently, 0–10% [3] of nuclear transfer is efficacious. Between weaning age and the age of four, cloned calves developed from somatic cells have an annual mortality rate of at least 8% [11].

1. **Transgenesis =** Transgenesis is the process by which one introduces a desired DNA segment or gene (transgene) to an animal so that it is capable of transmitting the transgene to all of its progeny.

An animal has been determined to be transgenic if a foreign gene has been introduced into its genome for the purpose of altering its DNA. Somatic cell nuclear transfer (SCNT), gene transfer into gametes, and the DNA microinjection technique are the three types of techniques utilized to transfer foreign DNA [5].

Nowadays, transgenesis has been employed in the majority of food animals, which comprises fish, cattle, sheep, goats, pigs, rabbits, and sheep. The first transgenic animal is a mouse. The first transgenic livestock, i.e., sheep, were developed in 1985 by microinjection of foreign DNA into one pronucleus of a zygotic (Hammer et al., 1985) [6]. For many years, microinjection was the technique of choice, even though more efficient methods based on somatic cell nuclear transfer (SCNT) are now available. At present, transgenic animals are not employed in breeding schemes, even though they are used to improve milk's protein content and develop proteins for therapeutic purposes.

1. **Biology of Pluripotent Stem Cells (PSCs) =** Pluripotent stem cells have the ability to self-renew and mature into the three primary germ cell layers, which eventually result in the development of all of the adult body's cells and tissues.

PSCs can be generated from two different sources: induced pluripotent stem cells (iPS cells), which can be generated by reprogramming somatic cells, and embryonic stem cells (ES cells), which are obtained from an embryo [7].

Induced pluripotent stem cells (iPSCs) are produced from two endangered species: Mandrillus leucophaeus and northern white rhinoceros [20].

**Application of Assisted reproductive technologies:**

1. Assisted reproductive technology is utilized to expand the lineages that are less common.
2. It is done in order to produce a large number of young, quality females.
3. By allowing female progeny testing, it is used for improving genetic research.
4. It shortens the generation gap.
5. It might assist in reintroducing genetic material to breeding populations.
6. The use of sires with superior genetic material is expanding due to technology similar to artificial insemination.
7. It might be useful for directly recovering gametes, such as oocytes and sperm, from an animal's gonads following death or gonadectomy.
8. The relative contributions of the aging oocyte and the aging reproductive tract to decreased reproduction in elderly animals are assessed using embryo transfer technology.
9. It is possible to transport frozen embryos across great distances, such as from one nation to another, utilizing cryopreservation embryo technology, which makes exporting cattle more affordable.
10. It is used to create twins in cattle by either implanting a single embryo into each uterine horn of an infertile cow or transferring a second embryo to the recipient cow that had given birth a few days previously using the embryo transfer procedure.
11. Any gene that is responsible for causing any abnormal conditions can be identified through marker-assisted selection [9].

**Reference:**

1. Bertolini, L. R., & Bertolini, M. (2009). Advances in reproductive technologies in cattle: from artificial insemination to cloning. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, *56*(III), 184-194.
2. Wakchaure, R., Ganguly, S., Praveen, P. K., Kumar, A., Sharma, S., & Mahajan, T. (2015). Marker assisted selection (MAS) in animal breeding: a review. *J. Drug. Metab. Toxicol*, *6*(5), e127.
3. Tian, X. C., Kubota, C., Enright, B., & Yang, X. (2003). Cloning animals by somatic cell nuclear transfer–biological factors. *Reproductive Biology and Endocrinology*, *1*(1), 1-7.
4. Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., & Campbell, K. H. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature*, *385*(6619), 810-813.
5. Shakweer, W. M. E., Krivoruchko, A. Y., Dessouki, S. M., & Khattab, A. A. (2023). A review of transgenic animal techniques and their applications. *Journal of Genetic Engineering and Biotechnology*, *21*(1), 1-14.
6. Niemann, H., Kues, W., & Carnwath, J. W. (2009). Transgenic farm animals: current status and perspectives for agriculture and biomedicine. *Genetic Engineering in Livestock: New Applications and Interdisciplinary Perspectives*, 1-30.
7. Kumar, D., Talluri, T. R., Selokar, N. L., Hyder, I., & Kues, W. A. (2021). Perspectives of pluripotent stem cells in livestock. *World Journal of Stem Cells*, *13*(1), 1.
8. Vikrama, C. P., & Balaji, N. S. (2010). Use of assisted reproductive technologies for livestock development. *Veterinary World*, *3*(5), 238.
9. Georges, M., Dietz, A. B., Mishra, A., Nielsen, D., Sargeant, L. S., Sorensen, A., & Womack, J. E. (1993). Microsatellite mapping of the gene causing weaver disease in cattle will allow the study of an associated quantitative trait locus. *Proceedings of the National Academy of Sciences*, *90*(3), 1058-1062.
10. Georges, M. (1999). Towards marker assisted selection in livestock. *Reproduction Nutrition Development*, *39*(5-6), 555-561.
11. Wells, D. N., Forsyth, J. T., McMillan, V., & Oback, B. (2004). The health of somatic cell cloned cattle and their offspring. *Cloning & Stem Cells*, *6*(2), 101-110.
12. Garry, F. B., Adams, R., McCann, J. P., & Odde, K. G. (1996). Postnatal characteristics of calves produced by nuclear transfer cloning. *Theriogenology*, *45*(1), 141-152.
13. Hill, J. R., Roussel, A. J., Cibelli, J. B., Edwards, J. F., Hooper, N. L., Miller, M. W., ... & Stice, S. L. (1999). Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology*, *51*(8), 1451-1465.
14. Kato, Y., Tani, T., Sotomaru, Y., Kurokawa, K., Kato, J. Y., Doguchi, H., ... & Tsunoda, Y. (1998). Eight calves cloned from somatic cells of a single adult. *Science*, *282*(5396), 2095-2098.
15. Kubota, C., Yamakuchi, H., Todoroki, J., Mizoshita, K., Tabara, N., Barber, M., & Yang, X. (2000). Six cloned calves produced from adult fibroblast cells after long-term culture. *Proceedings of the National Academy of Sciences*, *97*(3), 990-995.
16. Renard, J. P., Chastant, S., Chesné, P., Richard, C., Marchal, J., Cordonnier, N., ... & Vignon, X. (1999). Lymphoid hypoplasia and somatic cloning. *The Lancet*, *353*(9163), 1489-1491.
17. Walker, S. K., Hartwich, K. M., & Seamark, R. F. (1996). The production of unusually large offspring following embryo manipulation: concepts and challenges. *Theriogenology*, *45*(1), 111-120.
18. Young, L. E., Sinclair, K. D., & Wilmut, I. (1998). Large offspring syndrome in cattle and sheep. *Reviews of reproduction*, *3*(3), 155-163.
19. Laible, G., Smolenski, G., Wheeler, T., & Brophy, B. (2016). Increased gene dosage for β-and κ-casein in transgenic cattle improves milk composition through complex effects. *Scientific reports*, *6*(1), 37607.
20. Friedrich Ben-Nun, Inbar, Susanne C. Montague, Marlys L. Houck, Ha T. Tran, Ibon Garitaonandia, Trevor R. Leonardo, Yu-Chieh Wang et al. "Induced pluripotent stem cells from highly endangered species." *Nature methods* 8, no. 10 (2011): 829-831.
21. Vikrama, Chakravarthi P., and N. Sri Balaji. "Use of assisted reproductive technologies for livestock development." *Veterinary World* 3, no. 5 (2010): 238.
22. Loskutoff, N. M., P. Bartels, M. Meintjes, R. A. Godke, and M. C. Schiewe. "Assisted reproductive technology in nondomestic ungulates: a model approach to preserving and managing genetic diversity." *Theriogenology* 43, no. 1 (1995): 3-12.