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

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**Introduction**

Assisted reproductive technology is defined as the reproductive technology which is used for genetic improvement of livestock. Mainly four generation of assisted reproductive technologies (ART) is there for animals. Commercial application of the first three generations of ARTs i.e., 1) artificial insemination (AI) and cryopreservation of gametes and embryos, 2) multiple ovulation and embryo transfer (MOET) and 3) *in vitro* fertilization (IVF) procedures and gamete and embryo sexing is successful but the fourth generation of ARTs which comprises cloning by nuclear transfer (NT) of somatic or embryonic cells, genetic marker-assisted selection (MAS), stem cell biology and transgenic animals is not successful compare to first, second and third generation of ARTs. Third and fourth generation of assisted reproductive technologies (ART) has the potential to increase the reproductive rate of superior animals but their commercial applications have been limited.

Nowadays use of **fourth generation** of assisted reproductive technologies is increasing. The fourth generation ARTs are as follows:-

1. **Genetic marker-assisted selection =** Previously selection for breeding in livestock is done on the basis of superior phenotypes but in recent years genetic marker-assisted selection is used in combination with phenotype. Information on genomes of livestock becoming available which can be used to improve desired traits in breeding programs. Many traits are governed by several loci or chromosomal regions, each can contribute to the variation in the trait and hence are called quantitative trait loci (QTL) [1]. The process by which genetic marker is used to identify the desired loci that particularly affect the single desired traits gene and also quantitative trait loci (QTL)is called as genetic marker-assisted selection MAS) [10]. Genetic gain in MAS is depends on the size of the QTL [2]. For example, marker associate with tenderness in cattle are available in the market [1].
2. **Cloning by Nuclear Transfer (NT) =** Nuclear transfer cloning is a cloning technique in which somatic cell nucleus is introduced into an enucleated metaphase-II oocyte from which new individual generate and genetically identical to the somatic cell donor. In 1990s, Ian Wilmut, Jim McWhir, and Keith Campbell performed experiments while working at the Roslin Institute in Roslin, Scotland. They have done experiment in many sheep and those sheep born in July 1996 and from those sheep one sheep named Dolly born 5 July 1996. Dolly was the first sheep cloned and developed from the nuclei of fully differentiated adult cells, rather than from the nuclei of early embryonic cells [4].The challenges faced in somatic cell cloning is low efficiency and incidence of formation of developmental abnormalities is high [12-18].Currently the efficacy for nuclear transfer is 0-10 % [3]. The annual mortality rate of cloned cattle which is produced from somatic cell is at least 8% between weaning age and 4 years of age [11].
3. **Transgenesis =** The process by which desired DNA segment or gene (transgene) is transferred to an animal so that it is able to pass the transgene on to all of the offspring is called transgenesis. Transgenic animal is defined as an animal in which foreign gene is inserted into its genome to alter its DNA. Three types of technique are used for transfer of foreign DNA: DNA microinjection technique, gene transfer into gametes, and somatic cell nuclear transfer (SCNT) [5]. Nowadays, transgenesis is applied in most of the food animals like cattle, sheep, goat, pig, rabbit, chickens and fish. Mouse is the first transgenic animal. First transgenic livestock i.e., sheep were produced in 1985 by microinjection of foreign DNA into one pronucleus of a zygotic (Hammer et al.1985) [6]. For many years microinjection was the method of choice but now more efficient technique are available which is based on somatic cell nuclear transfer (SCNT).At present, transgenic animal is not embody into breeding programs but it is used to improve the protein content in milk [19] and to produce therapeutic protein in the milk.
4. **Pluripotent stem cells (PSCs) biology =** Pluripotent stem cells have self-renewal capacity and develop into the three primary germ cell layers which will result into formation of all cells and tissues of the adult body. There are two sources of PSCs one is embryonic stem (ES) cells which developed from an embryo, and another one is induced pluripotent stem (iPS) cells which is derived via reprogramming of somatic cells [7].

**Application of Assisted reproductive technologies:**

1. It is used to increase the less available bloodlines.
2. It is used to obtain numbers of offspring from valuable females.
3. It is used to increase the genetic progress by facilitating progeny testing of female.
4. It reduces the generation interval.
5. In case of Embryo transfer technology it is used to evaluate the relative contributions of the aging oocyte and the aging reproductive tract to decreased reproduction in older animals.
6. By using cryopreservation of embryo technique it is used to transport frozen embryo over long distance like from one country to another country which results in inexpensive means of livestock export.
7. It is used to produce twins in cattle, transferring either a single embryo into each uterine horn of an unanimated cow or a second embryo to the contralateral uterine horn of a recipient cow that conceived a few days earlier by using embryo transfer technique.
8. By using marker assisted selection it is used to detect any gene which is responsible for causing any abnormal disorders [9].

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