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LIVESTOCK PRODUCTION – EMERGING PRACTICAL APPROACHES

Review Based Book Chapter

ANIMAL BREEDING AND BIOTECHNOLOGY

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REVIEW BASED BOOK CHAPTER

ANIMAL BREEDING AND BIOTECHNOLOGY

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Abstract

Livestock plays a vital role in the global economy, sustaining the livelihoods of more than 1.3 billion people and providing roughly 34% of the world's dietary protein. Despite concerns about sustainability and environmental impact, there is an anticipated rise in demand for animal products in the up-coming years. Biotechnology is a vital technique in the field of animal breeding, as it plays a major role in feeding the world's growing population and rearing domestic animals. Biotechnological advancements in breeding technologies such as artificial insemination, semen cryopreservation, sperm sexing and selection, in-vitro fertilization have modernized animal breeding, enhancing the performance of livestock production. Continued advancement in cloning and transgenic animals also provides opportunities for increasing livestock production. Furthermore, consistency and quality in animal feed, fiber, and medical products can all be improved with the help of modern biotechnology. This chapter tends to explore the role of biotechnology in animal breeding and the importance in improving livestock productivity.

Keywords
Livestock, Biotechnology, Animal Breeding, Insemination, Transgenic Animals

Contents

7.1. LIVESTOCK ROLE IN ECONOMY OF COUNTRY

Data from the FAO show that livestock supports the livelihoods of at least 1.3 billion people globally and provides around 34% of the world's dietary protein, accounting for about 40% of agricultural output in developed countries and roughly 20% in developing ones.

The problem of feeding the globe in 2050 while protecting the environment and providing a healthy, balanced diet for everybody is enormous. While consumption appears to be declining or stagnating in some parts of the world, between now and 2050, there will likely be a rise in demand for main animal commodities worldwide. Over the next ten years, there should be a 15% increase in the global demand for meat, and by 2027, there will be a 25% increase in the consumption of milk and dairy products worldwide [1].

Animal food products supply essential nutrients to humans (much beyond simply protein!), which are unavailable from plants because of their lower content (or absence), reduced bioavailability, and/or the presence of anti-nutritional agents. Despite the fact that current diets are frequently out of balance, removing animal products from the human diet would restrict the amount of essential nutrition that populations get.

In addition to producing food, the production of animals serves a variety of other economic, cultural, and social goals. It also contributes significantly to the vitality of regions and is a crucial component of agro-ecosystems. Positive externalities related to livestock should and must be taken into account.

Worldwide, 14% of the dry matter consumed by cattle worldwide is fit for human consumption. Animals play a crucial role in turning the remaining 86% of the biomass—grass and agricultural residues—into extremely nutritious human food and manure, which serves as a source of carbon and nutrients for soils and plants. Essentially, this is a positive cycle.

On 57% of land that cannot be used for crops directly (marginal land), livestock husbandry provides food and ecosystem services. Producing animals—especially herbivores—helps ensure food security by bringing value to marginal grazing land that cannot yield plant items. Approximately 25% of the global animal product is produced by 600 million small ruminants and 360 million cattle on marginal terrain.

The following significant ecosystem services that grasslands and marginal areas provide have been identified and described: preservation of rural landscapes and biodiversity associated with grasslands; prevention of rural fires by controlling bush encroachment; preservation of rural populations with economic returns; and contribution of livestock outputs like manure, which has a high carbon to nitrogen ratio and positively affects soil organic matter content and macro-fauna (earthworms). Animal production can support the preservation, rehabilitation, and sustainable use of terrestrial ecosystems and the services they provide, fight desertification, repair degraded land and soil, and ensure that small-scale food producers have a means of subsistence, equitable access to markets, and opportunities that recognise the unique qualities of regional goods [1, 2].

It will not be feasible to discuss biodiverse ecosystems or sustainable, healthy food systems without livestock farming, animals, and animal products. Systems of animal production are crucial to the accomplishment of many sustainable development objectives and to the vitality of many different parts of the world.

7.2. ANIMAL BREEDING

The selective crossing of domestic animals with desirable traits to produce improved offspring is known as animal breeding. For certain intriguing features, these "qualities" are interpreted as breeding values. A few examples would be the quantity and quality of milk and meat produced, as well as disease resistance, robustness, fertility, longevity, and a decrease in environmental impact (e.g., better utilization of feed resources and a reduction in methane).

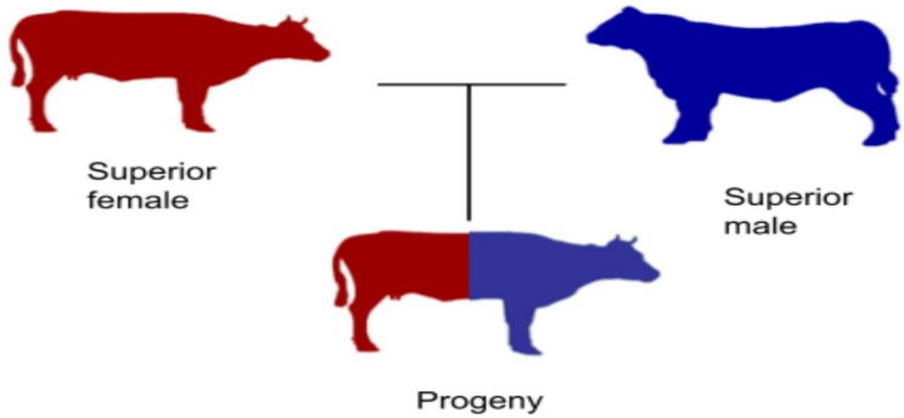

Figure 1: Animal Breeding is done by selecting animals having desirable traits

The objectives of animal breeding are

- To increase animal yield
- To improve the desirable quality of animal produce
- To produce disease-resistant varieties of animals
- Production of food products (e.g. dairy, meat, eggs)
- Production of non- food products (e.g. wool, leather)
- Sports
- Companions
- Maintaining rural areas
- Medical applications/research
- Preservation of minor breeds, e.g. Jacob sheep
- Reintroduction programs, e.g. golden lion tamarins

These animal breeds then use for four principle reasons

- as sources of usable products or services
- for medical or scientific research
- for aesthetic, cultural or ethical considerations
- and as pets [3]

The advancement of animal production methods, from conventional to organic, is significantly influenced by animal breeding. Entire populations can be improved by boosting an animal's capacity for a certain trait, which helps farmers, consumers, and the environment. Superior genes are accumulated and less desirable genes are eliminated through animal breeding. A breeding program selects parents with the objective of improving genetics for the offspring. The majority of the traits (production, reproduction, and health) studied in livestock animals have shown genetic diversity. The best animals are chosen as parents for the subsequent generation in breeding programs, which take advantage of this genetic variety to raise the population mean level. As the pinnacle of the animal production pyramid, livestock breeding establishes the standard for all animals utilised in agriculture.

In addition to increasing production, breeding of pigs, poultry, and dairy cattle has reduced greenhouse gas emissions per unit of animal product. Methane greenhouse gas emissions from cattle can be decreased by either utilising males with low methane emissions assessed or by choosing animals with higher feed conversion efficiency. Reducing greenhouse gas emissions and raising agricultural output can both be achieved by breeding for low methane emissions. Local breeds are also preserved, enhanced, and promoted by breeders.

In terms of growth rate, milk yield, and egg production, breeding for enhanced productivity over the past 50 to 60 years has been quite effective; but, it has also had detrimental effects on welfare and behavior [4]. The broiler chicken serves as an excellent illustration of this: Here, selection for growth has been quite successful over the last 50 years, raising the growth rate from 25 to 100 g daily [5]. However, this achievement comes at the expense of a rise in cases of sudden death syndrome, ascites, and lameness [6] demonstrated that employing slower-growing broiler hybrids—which reach slaughter weight in eight weeks as opposed to six—can help address a number of these welfare concerns [7]. Selection for higher milk yield in dairy cows has led to lower longevity and fertility, more leg and metabolic issues, and an increased risk of mastitis and other illnesses.

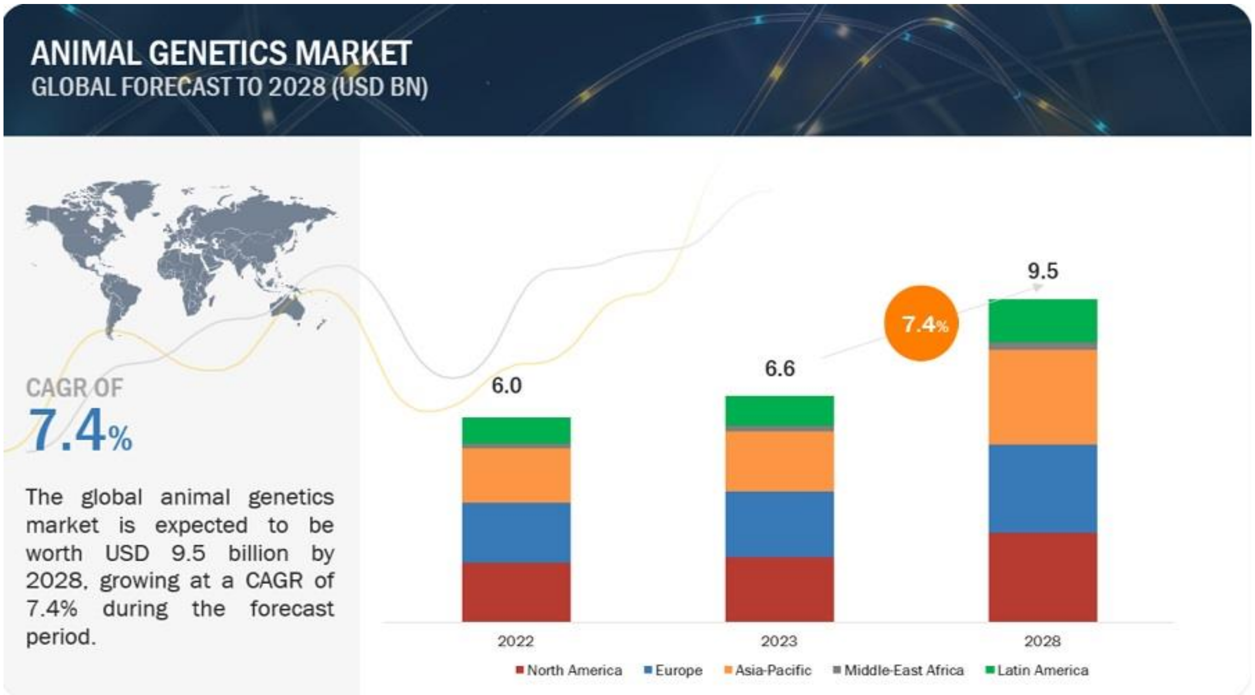

Figure 2: Animal genetics market (a global forecast) statistics shows increase in net worth by 2028 of hybrid animals

7.3. ANIMAL SELECTION

The selection of farm animals has a significant effect on the overall production of livestock since the breeding response is sustained and cumulative. The aim of selection is to generate superior breeding stocks that serve as the progenitors of subsequent generations. The process of selection enables the best animals to bear children and remove unwanted animals from the herd. The animals that are kept have some desirable characteristics that increase their productivity.

There are different kinds of selection, including sibling selection, progeny testing, individual or mass selection, and selection both within and between families.

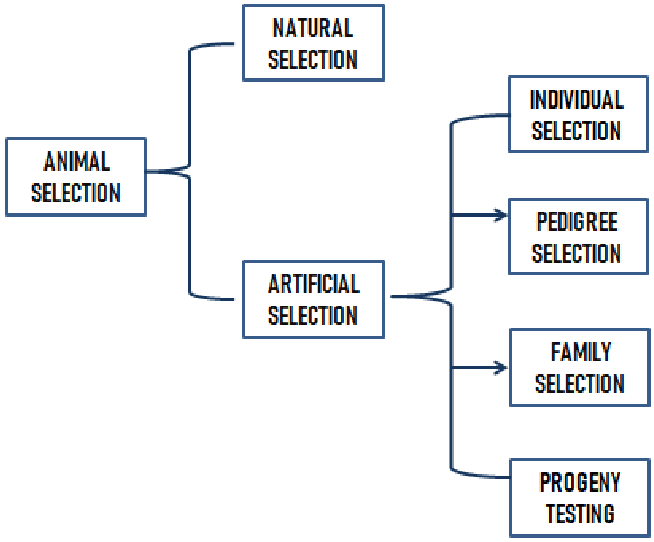

Figure 3: Types of animal selection

The best member of each family is used in family selection for breeding. The entire family is used in between family selection. Only the candidates' records are used in mass selection. When a trait is highly heritable and manifests early in life, mass selection works best; in these circumstances, observation and phenotypic-based selection are sufficient. Other selection techniques that involve relatives or progeny can be used alone or in combination when mass selection is not appropriate.

a. Progeny testing

Phenotypic evaluation of a parent's progeny is known as "progeny testing," and it is used in animal and plant breeding programmes to determine quantitative trait selection.

In the dairy and beef cattle industries, progeny testing is widely utilised to help assess and choose stock for breeding. When a high degree of accuracy is required to choose a sire to be widely employed in artificial insemination, progeny testing is most helpful. As mentioned in the section above, progeny testing programmes select the elite sires and dams in the population using an animal model evaluation. An excellent illustration is given by the explanation of progeny testing in dairy breeding as shown in Figure 4.

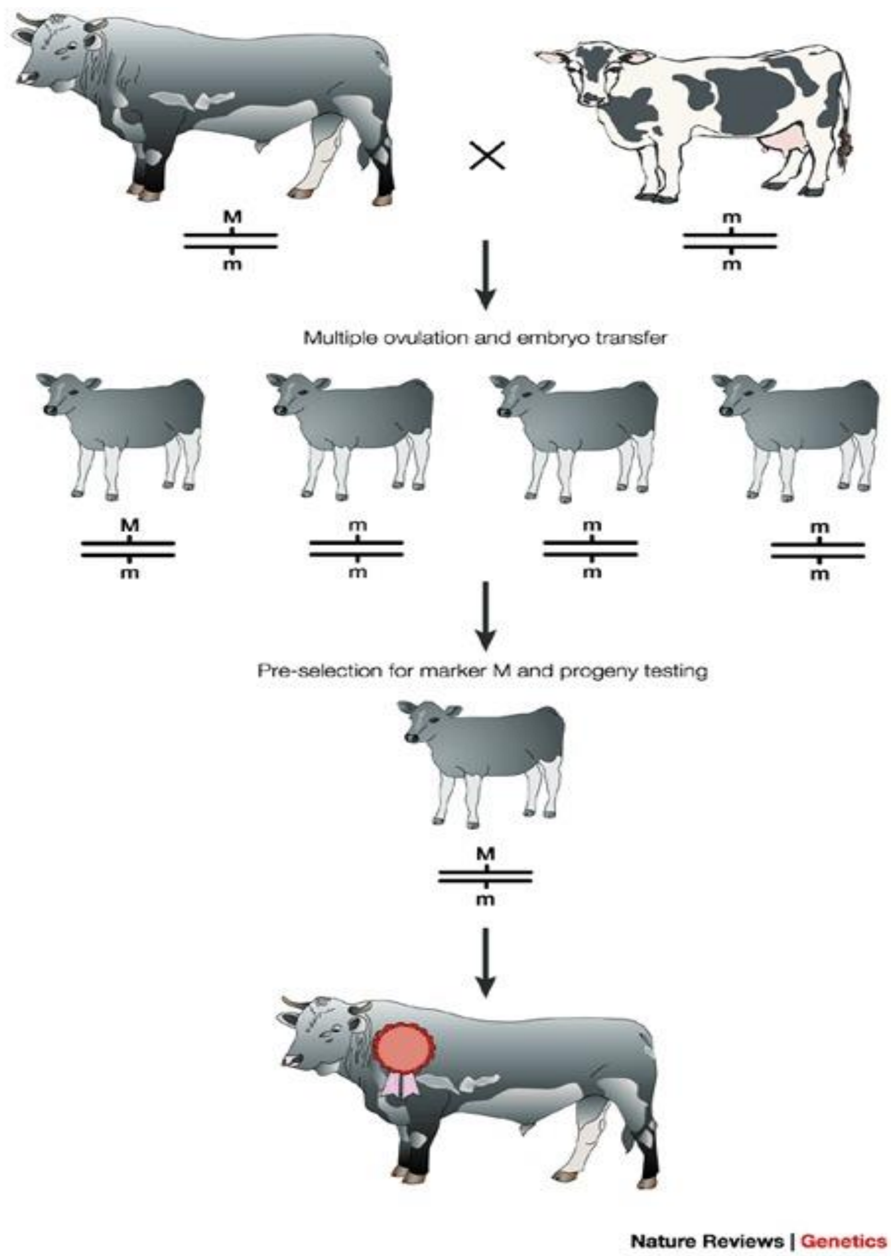

Figure 4: Marker-assisted pre-selection for progeny testing

Because milk production is a sex-limited trait, dairy bulls go through a progeny test, in which they are evaluated on the basis of the milk production of 60–100 daughters. After the progeny test, the best bulls are selected for widespread use in the population through artificial insemination. Because of the high cost involved, only a limited number of bulls can be progeny tested each year. Selection of bulls to be tested is based on ancestral information, which means that all members of a full-sib family have the same estimated breeding value. Molecular scores will, however, differ between full-sibs if they inherited different marker alleles. Through reproductive technology, such as multiple ovulation and embryo transfer, several bull calves are produced per female and selection of bulls to progeny test can be on the basis of molecular score37, 63. The combination of marker-assisted pre-selection and progeny testing has a greater chance of producing highly productive animals.

The top 1–2% of the population's cows is selected as bull moms, and the top bulls that have undergone progeny testing are selected to generate future generations of sires. Typically, bull moms' evaluation accuracy is around 40%, while sires that generate young bulls have evaluation accuracy of over 80%. The number of bulls utilised for artificial insemination is not as great as the business desires. In order to increase accuracy, each sire in the next generation is mated to 60–80 progeny, or enough cows in the population. Following their daughters' production history, the young sires are assessed, with the top 10% being heavily utilised in the production of commercial cows. It may take hundreds of daughters for some of the progeny-tested sires to find a better sire to take their position. These sires determine the genetic fate of dairy cattle because artificial insemination is used in the breeding of about 70% of these animals. It has been quite effective to use this selecting process consistently.

The best and most practical way to improve the genetic makeup of the breed is through progeny testing, which is available in many of the communities that make up its breeding tract and have access to AI services. The following is an example of a breeding design for a PT Programme that can be used in smallholder production settings.

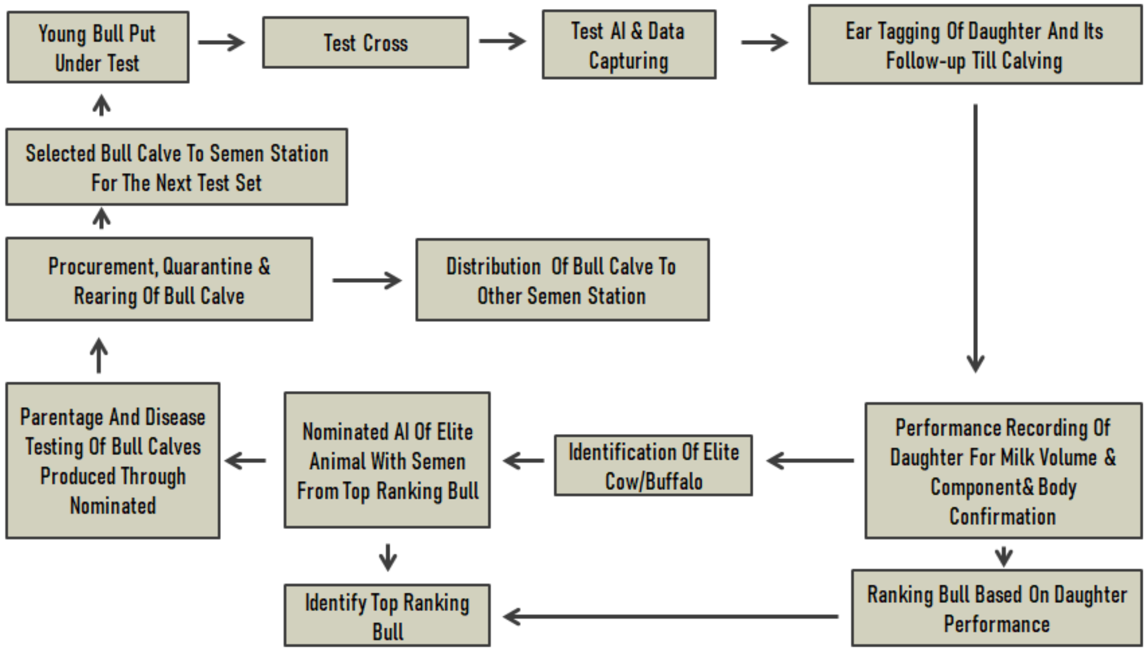

Figure 5: Progeny testing through young sire

7.4. BREEDING SYSTEMS

Systems of breeding can be classified into two major groups: Inbreeding and Out breeding [8].

a. Inbreeding

The mating of animals that are more closely related to one another than the average relationship within the population in concern is known as inbreeding. Up to 4-6 generations of shared ancestors should be present in the genealogy of the mated individual. Mating patterns like parent-child and brother-sister are examples of inbreeding. When related animals mate, their offspring share more DNA, which is why inbreeding is sometimes referred to as "narrowing the genetic base." Desired qualities can be concentrated through inbreeding. In general, domestic animals should avoid inbreeding. Higher death rates, slower growth rates, increased susceptibility to disease, and decreased fertility are all associated with increased inbreeding. Consequently, farmers attempt to steer clear of breeding similar animals.

There are two categories of inbreeding

- Line breeding
- Close inbreeding

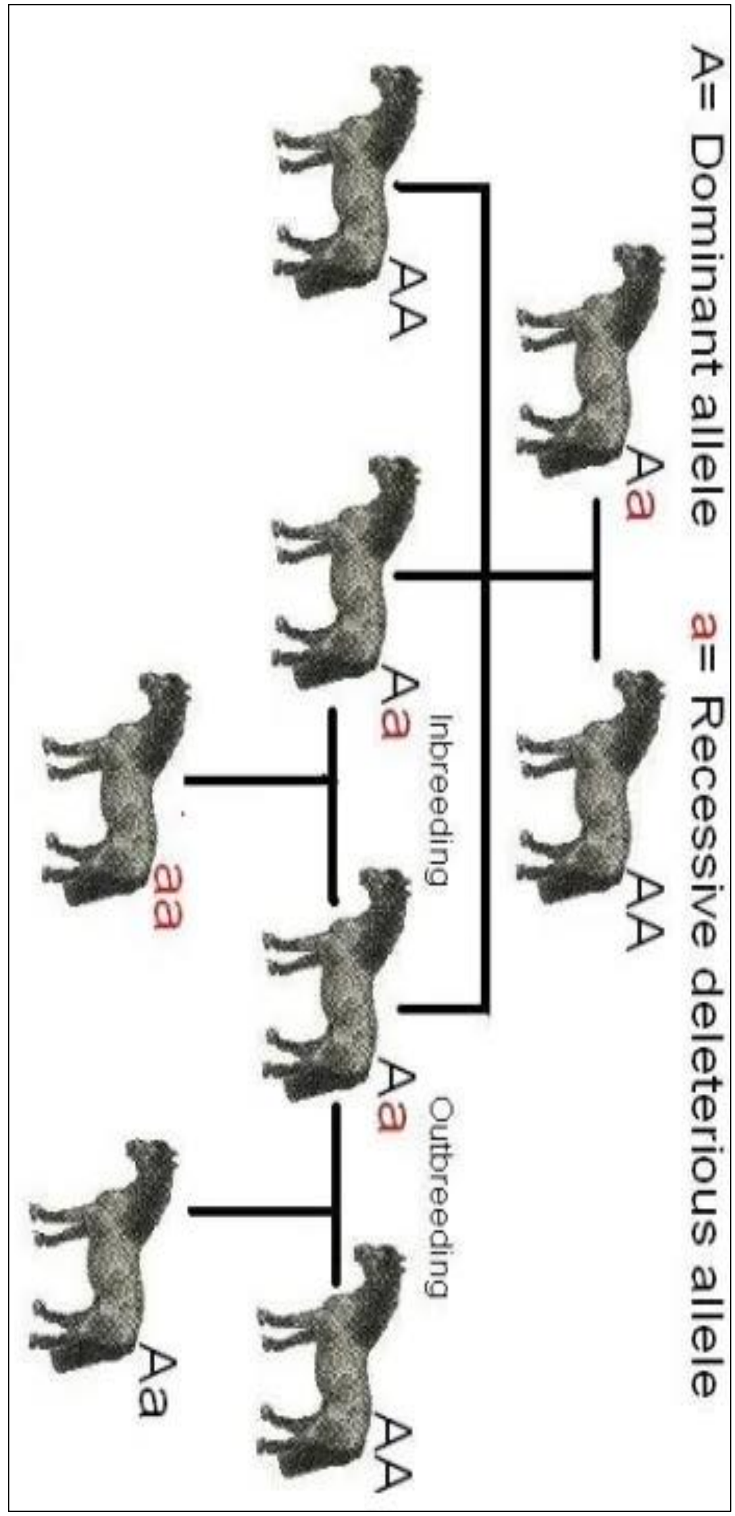

Figure 6: Inbreeding Vs Outbreeding

There are several mating between siblings or between parents and offspring to produce inbred lines with a comparatively high degree of homogeneity is known as close inbreeding. In line breeding, mated individuals maintain as close relatives to an ancestor as possible, resulting in a milder version of close inbreeding.

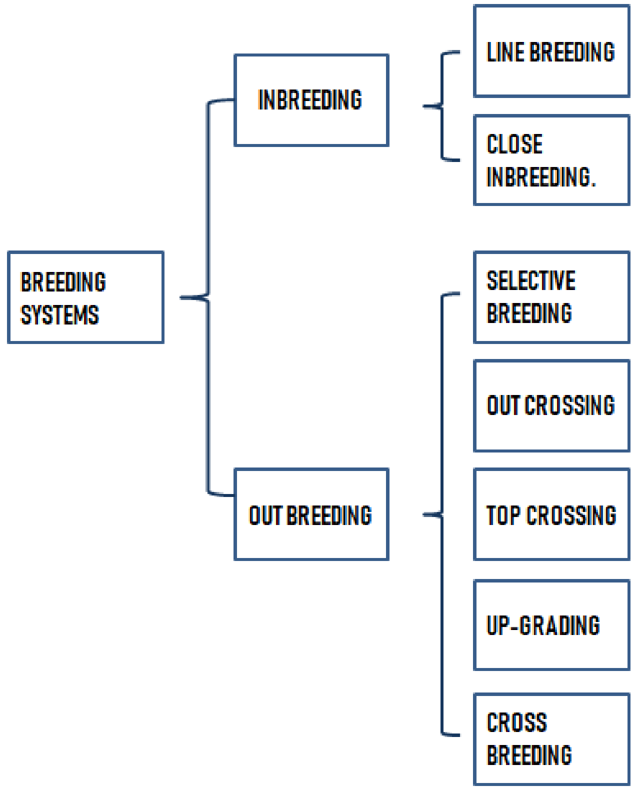

Figure 7: Different breeding systems

b. Out breeding

The mating of animals that are not as closely related to one another as the typical population is known as out breeding. Inbreeding has the opposite general effects from it. The individual's heterozygosity rises with outbreeding. Producing animals for the market is our breeding techniques' most practical application. In outbreeding, partners are selected solely on the basis of their lack of relatedness. In animal breeding, the following kind of outbreeding is employed:

1. Selective breeding

The process of mating individuals with desired qualities to increase the frequency of such traits in a population is known as selective breeding. The goal of selective breeding is to both develop and preserve the purity of the breed.

2. **Out crossing**

Outcrossing occurs when two lineages of the same breed are split apart for four or five generations and the father of one herd is employed in another herd. When there is genetic diversity and no selection response, it is employed. It introduces new genes in the population with reference - color, horn type, etc.

3. **Top crossing**

In a pedigree, top crossing usually refers to the best sire; it can also mean the continued use of sires to different families within a pure bred, same breed or different breed. Top crossing is the use of highly inbred sires to the dams of the base population or non-inbred population within the same breed.

4. **Up-grading**

Grading up or upgrading refers to the practice of consistently selecting one or more pure breed sires over nondescript population females. If sires from a specific breed (A) are back bred to nondescript animals or another breed (B) on a regular basis, there is a noticeable improvement in the crosses. It takes five generations to increase breed A's level of inheritance to 96.9% (0.969) in the fifth generation. Animals that have undergone five generations of backcrossing to a certain breed are eligible for registration as purebred after the conclusion of the fifth generation. A well-defined purebred population will be created from the nondescript population after seven to eight generations of continual grading up.

5. **Cross breeding**

Mating two individuals from different separate breeds is known as cross-breeding. Crossbreeding has been employed recently to create synthetic strains and new breeds e.g. Charolais and Angus meat cattle. For instance, crossbreeding Angus to generate high-quality meat with Charolais to produce particularly huge animals results in an animal that is both acceptable in terms of quality and size. When animals of different breeds are crossed, the progeny produce at a higher rate than their typical parents. There are several possible reasons for the increased production, including higher fertility, better mothering skills, faster and more efficient growth, and increased pre- and post-natal viability [8].

7.5. BREEDING BIOTECHNOLOGY

Biotechnology, defined as "Any technological application that uses biological systems, living organisms, or derivatives thereof to make or modify products or processes for specific use" [9]. It takes a lot of effort and skill to define biotechnology precisely. Consequently, there is a wide range of definitions, from very generic to highly restrictive, rather than just one. A general description of biotechnologies might be described as a collection of technologies that are founded on the transformation and utilization of living things to provide goods and services. Therefore, animal breeding alone qualifies as a biotechnology as its ultimate objective is the modification of animal germplasm to produce new generations of animals that are superior to existing ones for use in animal production [10].

Mankind made a significant contribution to the global population's development and advancement during the 20th century. Animal proteins can now be produced more effectively in the twenty-first century because to significant advancements in science and technology. Animal breeding and biotechnology play a crucial part in the efficient growth of the number of farm animals as well as the quality and quantity of animal products including meat, milk, and eggs. Genetics and reproduction are fundamental functional prerequisites for a successful animal production and are important elements of biotechnological research in livestock breeding. According to Smidt and Niemann [11], developed techniques that were used have a number of different aims in livestock breeding:

- Efficient use of production of sperm and ovarian cells
- Using advantages which has the frozen semen or embryo, primarily cattle, enabled the exchange of genetic material through the world
- Implementation of hygienic concepts of artificial insemination and embryo transfer use
- Improvement of genetic progress through integrated breeding programs based on the biotechnological procedures

- Improved and even completely new products obtained by domestic animals

The primary objective of creating and implementing innovative breeding techniques in animal husbandry is to give farmers access to the highest caliber reproductive male and female genotypes that are carefully chosen, together with a clear purpose and direction for production. Secondly, to try to maintain quality along the manufacturing chain at the lowest feasible cost while taking animal welfare, health, and ultimate product quality into account. It is possible to accomplish this goal in animal breeding through innovative methods and technologies in all domains by applying the most recent findings in genetic engineering, breeding, and reproduction. For many years during the last decade of the 20th century, new production techniques are developed in biotechnology, which have a specific application in practice and apply to the breeding of livestock [12] are given below:

- Artificial insemination and deep-frozen semen
- Application of embryo transfer and manipulation of the embryos
- Gender determination of semen and embryos
- Gene cloning and creation of functional gene structures
- Mapping genes
- Production of transgenic animals

7.5.1. ARTIFICIAL INSEMINATION

In artificial insemination, live sperm from the male are extracted and introduced in the female reproductive tract mechanically with the aid of instruments at a proper time. It results in production of normal offspring under most hygienic conditions. The first AI of domestic animal was on dogs in 1780 by Lazzaro Spallanzani [13].

In AI, the sperms are gathered from verified bulls (superior quality bull's germplasm), processed and then packaged into 0.25 or 5ml straws. Each straw contains significant amount of spermatozoa, somewhere between 9 and 30 million. A qualified veterinary physician uses an artificial insemination gun to insert these straws into the female reproductive system. Single ejaculation from the bull has the capacity to create over 200 straws.AI is most widely used ART in livestock during the 20th century, redefining and shaping the animal breeding sector. AI has been utilized in the bulk of domestic species encompassing dairy cattle, pigs over 80% and 90% respectively in Europe and North America. Now its utilization has been extended in horses, beef cattles, sheep, dogs, goat, deer, buffalo and humans. AI has frequently applied in conservation of rare and endangered species like elephants and primates.

Distinct methods of inseminations are utilized by various animal species. In cattles, the recto vaginal method of insemination is most secure and successful technique among speculum and vaginal method (Figure 8).

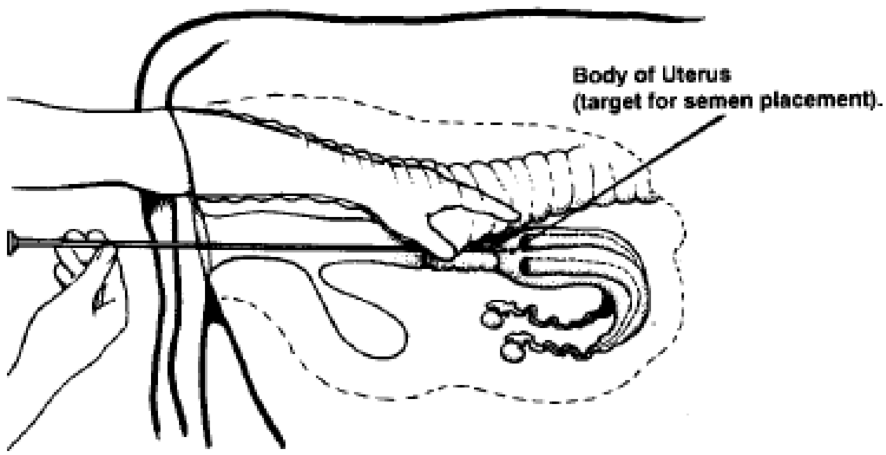

Figure 8: Position of artificial inseminations in cows

The AI practice serves core purposes within animal husbandry notably genetic improvement and its conservation, selective breeding, addressing infertility issues and their treatments. Utilization of elite bull in breeding programs results in successful propagation of high quality genetic traits through number of mating. Moreover, top-quality male semen can be preserved for prolonged durations in deep freezers, extending its useful life for up to 4 decades. For many farm animal operations, artificial insemination offers a workable substitute for natural breeding. Its main benefit is that it may be used to bring in genetically better sires, which will help the herd as a whole to enhance genetics.

AI offers several benefits over natural mating. These includes the eradication of need to upkeep a breeding bull, preventing the transmission of diseases, timely recognition of inferior male through routine semen examination aiding early progeny testing, facilitating the use of selected semen even after the sire's death. Moreover, AI allows for the shipment of semen between rural and urban areas allowing secure mating in animals of diverse proportion. AI assists in keeping breeding records, enhanced conception rates and even facilitates the use of aged, bulky and injured sires. AI has drawbacks in terms of including the need for competent staff and specialized equipment. It demands longer time than natural services and requires comprehensive knowledge of reproductive anatomy and physiology by the personnel. Improper sanitation of equipment and unhygienic conditions results into less fertility rates. Inadequate assessment of bulls can spread genital infections (viral and bacterial pathogen), potentially impacting the bull demand in market [14].

7.5.2. SEMEN CRYOPRESERVATION

Cryopreservation allows the cells and tissues to withstand low temperature with less or no metabolic activity. Semen cryopreservation is the collection, freezing and storage of sperm [15].

It is non-physiological approach that offers significant adaption to biological cells against osmotic and thermal stress. Theses stresses can occur throughout during the dilution, cooling-freezing and thawing procedures. Damage from freezing and thawing processes primarily affects cellular membranes, including the mitochondrial and plasma membranes, and in the most severe scenario, the nucleus. Specifically, the semen cryopreservation undergoes variety of oxidative, metabolic and physical abnormalities to sperm membrane. The membrane damage has an impact on loss of motility, change ATP content in spermatozoa and alteration in spermatozoa integrity having a great influence on fertility and viability. Temperature reduction, increased solute concentration, gas bubble formation, dehydration, increased ionic concentration; changes in pH, direct contact between cells, and precipitation of salts are the number of stress encountered. The majority of damage occurs at the onset and completion of protocols concurrently with the addition and removal of cryoprotectants. To minimize this damage, traditional strategies utilize additives, including cryoprotectant agent (CPA) like glycerol, along with antioxidants, fatty acids, sugar, egg yolk, milk, bovine serum albumin, liposomes, amino acids, membrane stabilizers. Cryoprotectants have suitable PH, osmolarity, buffering capacity that shields sperm cells from freezing damage (Figure 9). The first ever effective freezing of buffalo semen was reported by [16]. The first person to report a pregnancy with frozen-thawed buffalo bull spermatozoa was Basirov [17]. Conception rate in buffalo with the frozen thawed semen is 30%-60%.

Semen cryopreservation is important for understanding the benefits of AI, permitting the long term storage that arrest sperm metabolic activity without fertility loss. The discovery of glycerol cryoprotective abilities has made the preservation process achievable. Cryopreservation has a substantial effect on genetic improvement and disease mitigation in livestock, specifically in cattle industry. It has empowered producers by the means to acquire superior genetic stock at a possible reduced cost, in contrast to buy a bull, contributing to enhanced breeding efficiency in dairy and equine industry. However, the establishment of reliable cryopreservation techniques is important for preserving significant animal genetic assets. Sustaining sperm fertility is important due to acceptance of artificial breeding programs. Moreover, cryopreservation helps in storing animal genetics for allele variation, preserving rare and endangered species, providing benefits for genome resource banking. The use-age of frozen semen especially helps to restrict the spread of infectious pathogen, protecting the herd's health. By the distribution of agriculturally favorable genes, cryopreservation possesses its benefits in the increasing herd productivity. Cryopreserved semen could also lessen the burden of unexpected disaster or infectious disease epidemic. Reproductive research with non-domestic animals those involving the cryopreservation of sperms, oocytes and embryos also provide insight in establishing more genetic and conservation programs. Cryopreservation of boar semen is essential for facilitating international sales, ultimately benefiting economy [15, 18, 19].

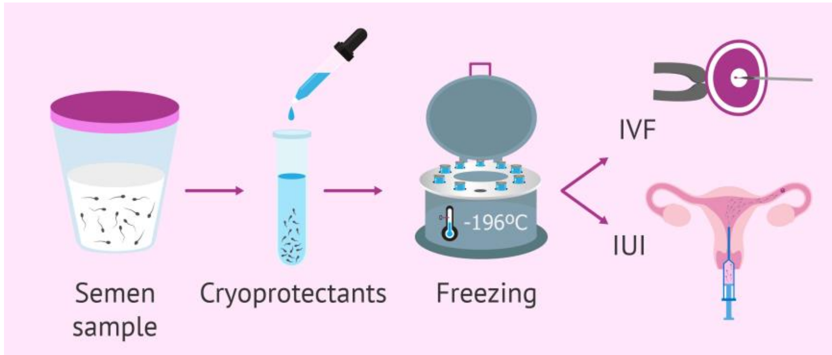

Figure 9: Usage of cryopreserved semen

7.5.3. SPERM SELECTION

Artificial insemination (AI) and sperm cryopreservation are two examples of modern biotechnologies that have completely transformed the animal breeding sector with the sperm selection being a crucial step in ART to improve the chances of successful fertilization and pregnancy.

There are various ways for selecting sperm in nature. Some rely on the characteristics of the sperm that are self-selective, while others use the female reproductive canal to impose a selection. Motility, morphology, chromatin integrity, capacity for capacitation, and acrosome reaction are the sperm characteristics that are taken into consideration in this selection. About 10% of all ejaculated spermatozoa enter the cervix, 1% enters the uterus, and 0.1% enters the Fallopian tube as a result of this selection. Pre-freeze sperm selection, however, is only done for artificial insemination programs and is based on sperm motility and concentration in the ejaculate, which still contains aberrant and dead sperm cells. These cells are an inevitable source of reactive oxygen species (ROS), which cause lipid peroxidation to destabilize the sperm membrane. Such oxidative stress is harmful to the acrosome, plasma membrane, and viability of sperm. Therefore, before processing semen ejaculate for cryopreservation, it is now essential to remove dead or malformed spermatozoa, which are an additional source of ROS. It is crucial to note that eliminating these ROS sources enhanced the quality and fertility of the post-thaw state in cows.

Based on natural sperm selection in the female reproductive tract, many biomimetic procedures have been created that select high-quality spermatozoa and decrease the amount of dead, moribund, and weakly motile sperm from the cell suspension. The swim up approach is one of these processes that choose spermatozoa based on sperm motility and morphology, whereas filtration involves sperm motility in addition to their interaction with filter substrates, such as glass fibers, SephadexTM beads, or membrane pores (Figure 10). For normal azoospermic semen quality with a high sperm count and decent motility, it is advised. Additionally, it is performed in a 45-degree angle round-bottom tube and promotes the selection of motile sperm with intact membranes, increasing the clinical pregnancy rate in the IVF lab [20, 21].

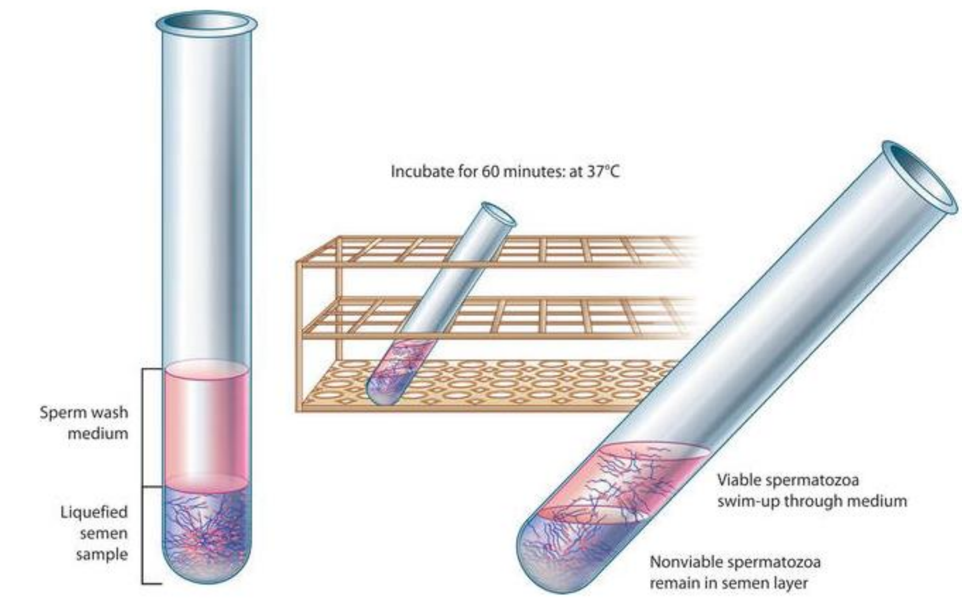

Figure 10: Swim up method

In Simple wash method, the sperm pellet and seminal plasma were separated using one or two centrifugations of the semen sample. Sperm count, normal morphology, and motility are not dramatically reduced by this technique; rather, the post-washed sample contains more sperm cells with rapid forward advancement and hypermotility. Additionally, this treatment is used for asthenozoospermic semen samples and severe oligospermia cases. It is advised for ICSI insemination rather than the normal IVF insemination procedure (Figure 11) [21].

Figure 11: Sperm washing through centrifugation

In density gradient centrifugation the spermatozoa are separated according to their density and each spermatozoon is discovered at the gradient level corresponding to its density at the conclusion of each centrifugation. Additionally, the density of a typical morphological spermatozoon is at least 1.10 g/mL, but an abnormal one has a density of 1.06–1.09 g/m. Furthermore, at the interphase between seminal plasma and 45% and 90% leucocytes, cell debris, and sperm cells with aberrant morphology and poor motility are discovered after centrifugation. Additionally, the pellet at the tube's bottom is made up of live, morphologically normal, and extremely motile spermatozoa. Additionally, because the spermatozoa must move a shorter distance between layers, a larger number of motile spermatozoa can be recovered if the volume of each gradient is smaller than 1 ml (Figure 12). This technique is suggested for insemination IVF or ICSI and is used for norm-azoospermia semen and poor semen quality [21].

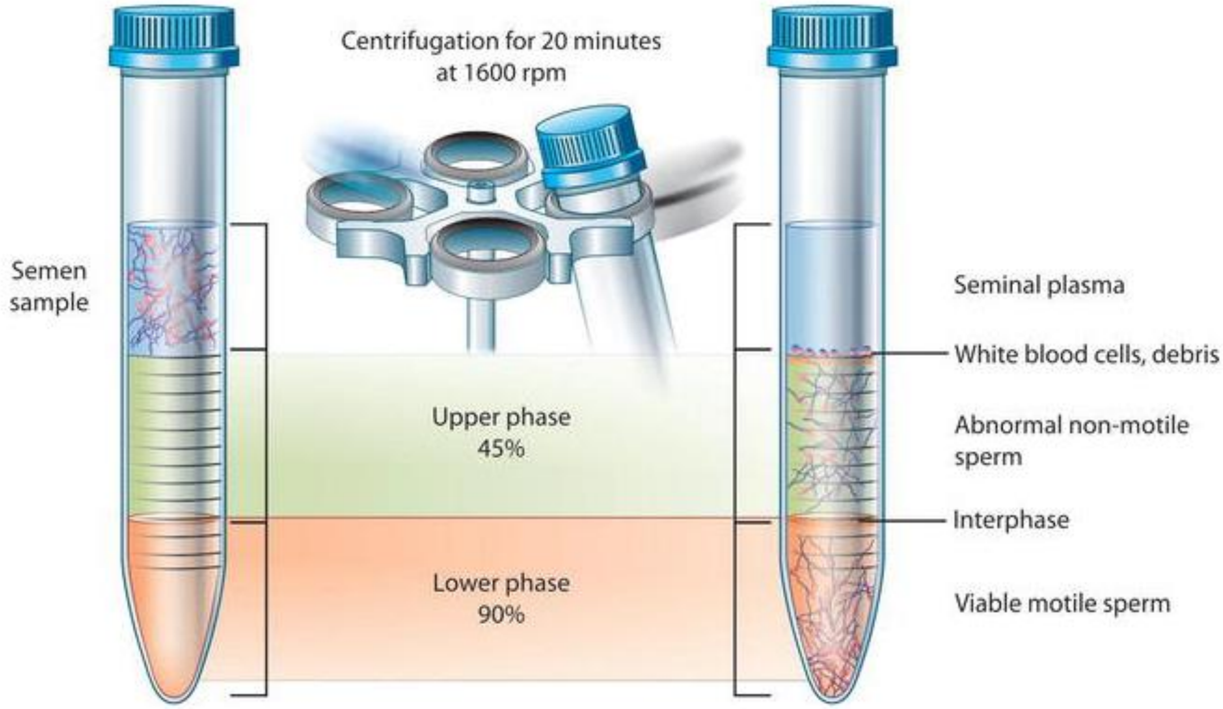

Figure 12: Density gradient centrifugation process

This procedure has the advantage of removing most leucocytes from the ejaculate, being quick and simple to transport in sterile conditions, and taking less time. However, the main drawback is that the spermatozoa obtained using this method has less DNA integrity than spermatozoa obtained using the swim-up method [21].

Zeta potential, it is the electrical charge potentials that are present between the membrane surrounding the sperm and the negatively charged sperm. The negatively charged sperm membrane is caused by the epididymal protein that is present on its surface and in sperm cells with damaged DNA, this is reduced (Figure 13).

Additionally, the procedure is simple to use and affordable. This method can be used to separate sperm with intact DNA, superior motility, and normal morphology. In IVF, sperm with negative zeta potentials had a higher fertilization rate of 65.79% when compared to sperm picked using double density gradient centrifugation. Additionally, in a randomized prospective study, there was a higher rate of fertilization and potential pregnancy among infertile couples with male factor infertility whose semen sample was selected with double density gradient/zeta potential as opposed to double density gradient alone during an ICSI procedure [14].

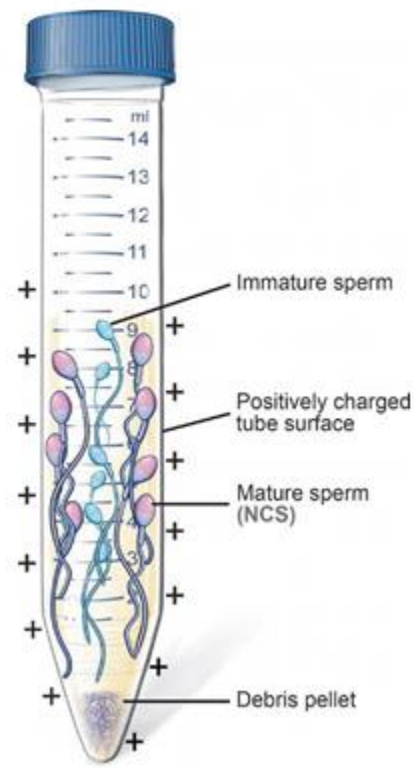

Figure 13: Charge based sperm selection

Sperm selection benefits by

- increasing the "shelf-life" of in vitro stored sperm samples
- improving sperm quality in AI doses
- removing pathogens
- improving cryosurvival by removing dead and dying spermatozoa prior to cryopreservation or used post-thawing to select the live spermatozoa
- to choose spermatozoa with acceptable chromatin integrity and normal morphology for AI, ICSI, IVF hence raising the number of zygotes that reach the blastocyst stage
- in addition to other sperm sexing techniques, to expedite the procedure [21].

Table1: Different methods for sperm selection for IVF and ICSI

Method	**Advantages**	**Disadvantages**
Simple wash	It is easy to do and yields a large amount of spermatozoa	increase in spermatozoa DNA damage
Density gradient	• It can be completed quickly and easily in a sterile environment • The majority of leucocytes in the ejaculate are eliminated by it	Endotoxin contamination is a risk factor The DNA integrity of sperm cells recovered with this approach is poor
Swim up	• High DNA integrity spermatozoa are found • It is inexpensive and easy to use	The preparation time is increased
MACS	There is a molecular level involved It is quick, easy, and non-invasive It distinguishes and divides apoptotic and non-apoptotic spermatozoa	It is used in conjunction with a density gradient to eliminate materials such as apoptotic spermatozoa, leucocytes, and seminal plasma
HA	In a severe oligozyospermic sample, it facilitates the selection of sperm cells with good DNA integrity	It is carried out in combination with a straightforward wash procedure and intracytoplasmic sperm injection (ICSI)
Zeta potential	It is simple to do and doesn't cost much retrieval of spermatozoa with perfect motility, undamaged DNA, and normal morphology	It functions in tandem with the density gradient process

Microfluidic device	• It uses a tiny amount of semen • Recovered spermatozoa show a notable reduction in DNA fragmentation	IVF for humans has not yet been completed, yet pig spermatozoa are

7.5.4. SPERM SEXING

The FAO has acknowledged that production of pre-sexed livestock by sperm or embryo sexing when paired with other biotechnologies like proteomics, genomics such as sperm-mediated gene transfer presents a promising breeding strategy to address and meet up the demand for food production. Sperm sexing or semen sexing is one of recent innovations in reproductive technologies within livestock.

Livestock farmers consistently strive to produce offspring of desired sex to under control the rising demands for milk and meat, ultimately providing benefits to farmers. Separation of X and Y sperm for pre-selection of desired sex produces the optimal proportion of males and females. This is important as female cattles are pre-requistes for dairy industry males are important for beef industry. Regulation of sex proportions offers significant benefit to livestock industry facilitating enhanced management of food production, improvements in animal welfare, accelerating genetic selection and reduction in environmental impact. Early sex determination can save management costs by allowing for the selective management of exceptional bulls or cows [22, 23].

One can create desired-sex calves. It is possible to ensure a 90:10 females to male ratio or the opposite. Dystokia can be minimized by preventing the production of male calves.it increases the usefulness of genetic markers and decreases the cost of embryo transfer and progeny testing procedures. Genetically superior dairy females utilize fewer sexed sperm for heifer replacement.

The X and Y sperm have previously been separated using a variety of methods based on differences in surface charges, mass, size, swimming behavior, immunological structure, and other factors. While some indicated promising effects but lacked scientific support, others were still up for debate. However, only flow cytometry is currently useful. 90% of the desired sex is consistently present in the semen that is processed using this method (Figure 14). However, despite the high accuracy, the process moves slowly and produces little. Furthermore, the equipment is very costly, and in order to ensure sorting accuracy, specifically trained technicians are required. Despite these drawbacks, commercial cattle usage of sexed semen production typically followed by cryopreservation for AI continues [24].

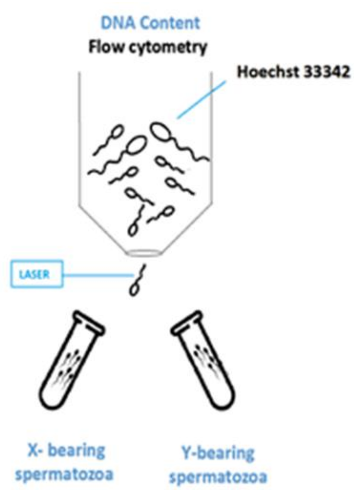

Figure 14: Sperm sexing through Flow cytometry

7.5.5. IN-VITRO FERTILIZATION

In-vitro fertilization is referred to as IVF. It's among the forms of assisted reproductive technology (ART) that are more known. In order to help sperm fertilize an egg and aid the fertilized egg's implantation in the uterus, in IVF employs a mix of medications and surgical techniques.

The methods for small ruminants and cattle IVF are well-established. Tyrode-derived saline supplemented with lactate, pyruvate, and bovine serum albumin together with a capacitating agent is the medium used for IVF. The female vaginal tract experiences physiological sperm capacitation. This phenomenon entails spermatozoa membrane remodeling as well as spermatozoa motility modifications that result in spermatozoa fertilization capacity. In vitro sperm capacitation is supported by numerous procedures. Heparin is frequently used for this purpose in cattle, typically without pretreatment. Spermatozoa in small ruminants are typically capacitated by an initial serum treatment obtained from females during oestrus. In order to enhance the sperm suspension with live, motile spermatozoa, frozen semen is typically utilized. Spermatozoa are placed at the bottom of the culture medium-containing tube and allowed to swim in it in the swim-up procedure. A specific incubation period is subsequently followed by the collection of the top fraction, which contains motile sperm. Alternatively, centrifugation in a discontinuous density gradient can be used to purify motile sperm (Percoll). After centrifugation, the motile fraction is collected at the tube's bottom. IVF uses sperm concentrations ranging from 0.5 × 106 to 2 × 106 mL−1 spermatozoa in fertilization medium. The standard co-incubation period for sperm and oocytes is 18–24 hours; however, a 6-hour incubation period appears to be adequate to achieve the higher level of proper fertilization (Figure 15) [25].

Live births following IVF were first shown in rabbits in the 1950s, then in mice a decade later. The livestock IVP market is growing quickly right now and doesn't appear to be slowing down. In 2017, about one million transferable in vitro-generated cattle embryos were produced worldwide, according to the International Embryo Technology Society (IETS). Sadly, not every nation provided data for this report, therefore the figures are underestimated. Since 2016, a notable surge has occurred, with only approximately 500,000 bovine embryos generated. The majority of the transfers took place in Brazil, with the USA coming in second. Approximately fifty thousand IVP embryo transfers were performed throughout Europe, with the majority occurring in Russia, then the Netherlands, Spain, Germany, and France. The total number of embryo transfers (both of IVP and in vivo-derived embryos) all over the world was over 1.5 million in 2017.

Livestock utilize a biotechnological procedure called IVF extensively. IVF has several benefits for cattle, but one of the main ones is the ability to multiply superior germplasm—animal genetic material that possesses desired qualities like high milk output, disease resistance, or other traits that are economically significant. Long-term clinical and scientific advancements in animal duplication have led to the creation of a range of instruments known as Assisted Reproductive Technologies (ART). These instruments are primarily designed to increase the number of progeny from genetically better animals and transfer germplasm to farmers and breeders. Moreover, ART makes it possible to effectively use donors who have anatomical impairments and sub-fertile circumstances to protect the germplasm of endangered species, home breeds, and transmission. Today's most significant developments in In-Vitro Embryo Production (IVEP) aim to enhance overall performance across the board, including ovarian stimulation, oocyte recovery, maturation, fertilization, embryo development, freezing, transfer, and pregnancy establishment. For the sake of future genetic security, native breeders are interested in preserving their native hybrids. In addition to preservation, conservation involves managing a breed for future use and improving its genetic potential. Identification, characterization, assessment, documentation, and conservation make up the strong control of animal assets. Farmers and animal breeders are utilizing IVF-Embryo transfer (ET) facility to multiply embryos from genetically superior animals. It enables rapid dissemination of desirable traits in the population. For those who are interested in producing desired sexed calves, during IVF only, sex-sorted semen is used. Nowadays, breeders are also trying to ensure genetic improvement in their herds, leading to higher productivity and profitability. As there is less transportation of live animals, it reduces the risks of disease transmission [26].

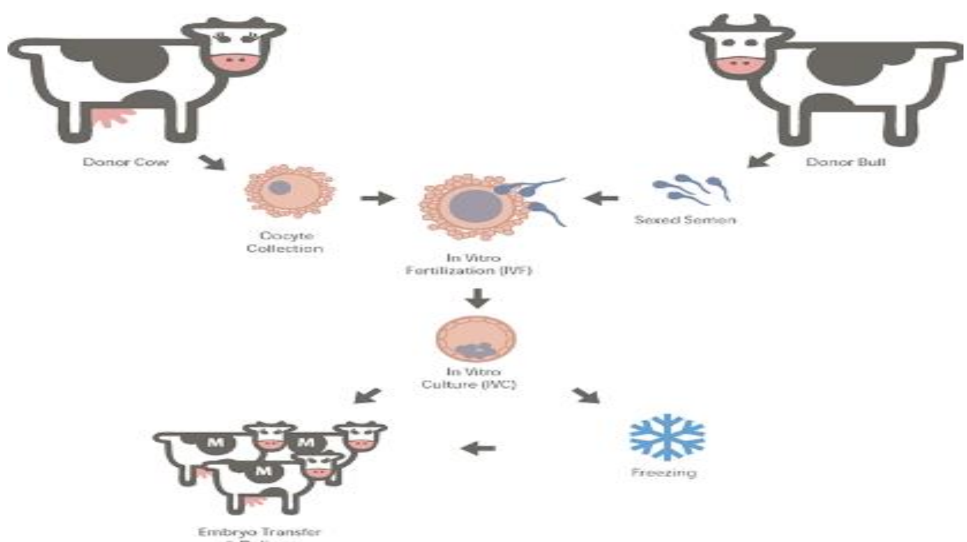

Figure15: Process of in vitro fertilization

7.5.6. EMBRYO TRANSFER

After AI and IVF, embryo transfer aims at the next technique aimed at a better control of animal reproduction. The development and application of contemporary reproductive technologies has created a wealth of opportunities for the investigation, treatment, and manipulation of the in vitro and in vivo reproductive phenomena, with the goal of enhancing the reproductive capacities of domestic livestock species. The procedure known as embryo transfer (ET) involves removing an embryo from a donor female and transferring it into a recipient female in order for the embryo to finish developing. From the standpoint of assisted reproduction history, Walter Heape successfully transferred the first set of mammalian embryos in 1890. Umbaugh reported the first successful embryo transfers in cattle in 1949. Through the transfer of cattle embryos, it became pregnant four times, but all of the recipients aborted before the pregnancies came to term. The first calf from an embryo transfer was born in 1951 when a day-5 embryo taken from a slaughterhouse was surgically transferred. Although producing only a fifth of the world's milk and a quarter to a third of its meat, developing nations are home to over two thirds of the world's cattle population. High productivity can be attained with the aid of new technologies, but for them to have an effect, producers must receive them. A cow typically gives birth to 8–10 calves during her lifespan. However, in contrast to the traditional breeding process, which requires the farmer to wait twelve months for a calf that may be either male or female, ET allows for the production of thirty-two embryos per cow annually.

In ET, the foundation for donor selection has been the selection of superior genetic or phenotypic animals. Since males are typically bred to numerous females and may be picked more precisely than females, choosing the male is actually typically more significant than choosing the donor female. Similar to this, choosing fertile bulls and fruitful semen is essential, which emphasizes the need of using high-quality semen. The recipient is one of the most crucial yet undervalued aspects of a successful ET program. Recipient possibilities are cows with good milking and mothering qualities, ease of calving, and robust reproductive systems. They have to be receiving the right kind of nutrients. Additionally, these cows need to be on a good herd health program. The receiving female's reproductive system should have parameters that closely mimic the donor's in order to enhance embryo survival after transfer. This necessitates the recipient and donor synchronizing their estrous cycles, ideally within a single day of one another. The recipients and donor cows can be synchronized in a similar way and at the same working time. Prostaglandin F2a (PGF2a)-synchronized receivers need to be treated 12 to 24 hours prior to donor cows, as PGF2a-induced estrus will manifest in the recipients in 60 to 72 hours and in donors who were super ovulated in 36 to 48 hours (Figure 16). The female bovine ovaries produce several viable, mature oocytes that can be fertilized in vivo and go on to grow into embryos. The actual embryo transfer procedure is comparable to artificial insemination, with the exception that the transfer gun is inserted far up the ipsilateral uterine horn to the CL. Six to eight days after breeding, the donor may be artificially or naturally inseminated, and the embryos will be harvested without surgery. Before being transferred, embryos need to be identified, assessed, and kept in an appropriate medium after collection. They might also undergo further manipulations at this point, like splitting and sexing, and they might be frozen or cooled for longer storage times.

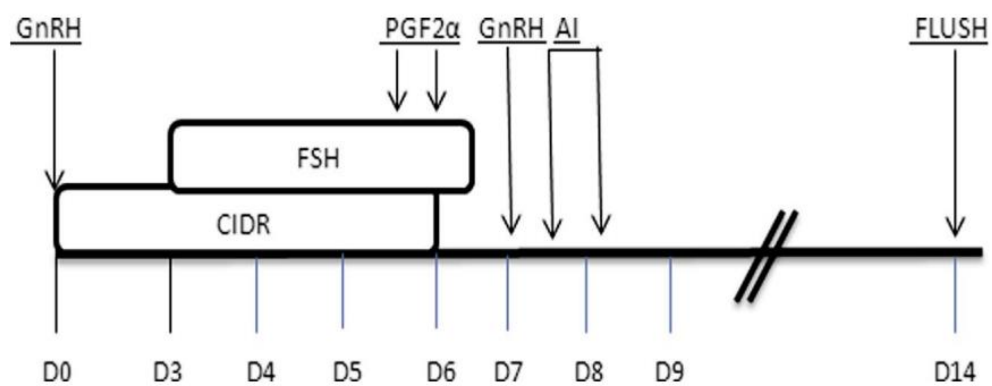

Figure 16: CIDR protocol

Increased selection intensity with ET helps genetic gain within herd commonly resulting in MOET (multiple ovulation and embryo transfer). Nucleus herds are currently being established in a number of nations, with male offspring being selected for the next generation of AI bulls and female offspring undergoing "Juvenile MOET". It has been estimated that genetic gains can quadruple in this fashion. However, it has been calculated that producing roughly six children per donor cow might increase the strength of selection and the rate at which qualities like growth that can be assessed in both sexes respond to genetic selection. Through superovulation and ET, it is possible to produce progeny from genetically valuable cows that have lost their fertility as a result of illness, injury, or aging [27, 28].

In animal production systems, the most popular application of embryo transfer is to multiply so-called desirable phenotypes. Embryo transfer offers the chance to distribute the genetics of tested, exceptional females, just as AI has made it possible for the genetic potential of a male to be widely disseminated. Additionally, ET allows herds of genetically important females to grow, helping to quickly increase a small gene pool through embryo transfer. Planned mating is most frequently used to produce AI bulls by embryo transfer.

Through the use of embryo transfer technologies, disorders like syndactyly can be prevented from spreading widely by screening young bulls for undesired recessive Mendelian traits prior to the distribution of semen for artificial intelligence. Additionally, only normal embryos can be transplanted after biopsied and karyotyped from parents whose karyotypes are abnormal. As a result, it has been proposed that ET be utilized to preserve genes during a disease epidemic, which may be a helpful substitute for creating herds free of disease. ET facilitates in the transportation of the diploid genome from cultured or frozen embryos with a lower risk of disease from one nation to another. With superovulation and ET, most cows may be tested with one or two sessions of superovulation; additionally, recipients can be twinned and the fetuses checked at roughly two months of gestation to diagnose many of these problems meanwhile Increasing the number of calves produced per gestation. Thus, an otherwise unsolvable issue may have a prompt solution through embryo transfer.

ET poses disadvantages in term of its cost; animals frequently die from illness, possibility of spreading an unusual illness, intricate logistics of transportation, minimal direct genetic impact in the event that females are imported. The success of other technologies that need to manipulate the oocyte or embryo in vitro depends on the use of effective embryo transfer procedures. These technologies include in vitro fertilization (IVF), sexual reproduction, creation of transgenic animals, embryonic bisection and nuclear transplant cloning [27, 29, 30].

7.5.7. CLONING

Cloning is the latest advancement in assisted breeding within animal husbandry. cloning does not alter an animal's DNA or modify its genetic composition. Through cloning, livestock producers can produce a genetically identical duplicate of an existing animal basically, an identical twin. Superior breeding animals called clones are employed to generate healthier progeny.

The world at large and the scientific community were fascinated by the first successful cloning of an adult Sheep-Dolly, the sheep that was cloned from a cultured mammary gland cell of a deceased 6-year-old ewe (Figure 17). The survival of the final Enderby Island cattle breed cow in Australia is a testament to the power of cloning. Cloning is utilized in the early 21st century to replicate the highest-producing dairy cows.

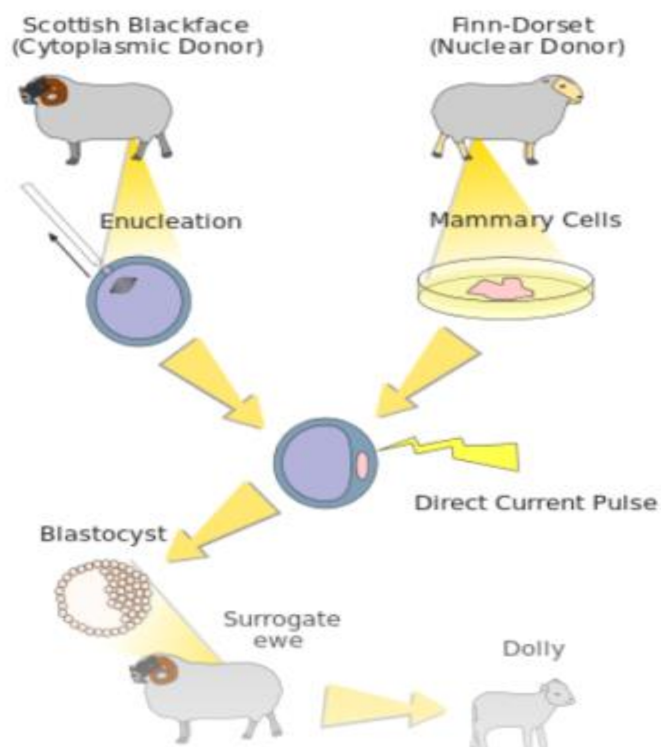

Figure 17: Cloning

The US Food and Drug Administration (FDA), has reassured the safety of animal clones and their progeny's meat and dairy products for human consumption. As they proceed toward commercialization, cloning firms will continue to negotiate a smooth marketing transition with the food sector and pertinent governmental organizations, such as the FDA and the USDA. The USDA announced on January 15, 2008, that there are no restrictions on the entry of cloned animal progeny into the food chain and that they are safe. The United States is currently adopting cloning very gradually.

Cloning has its internal acceptance. In the US, cloning livestock and selling cloned animals are safe and legal practices that have always existed. Argentina, Australia, Brazil, China, France, Germany, Iran, Japan, New Zealand, Turkey, and the United Kingdom are all engaged in cloning research. Foods derived from cloned animals and their progeny are safe, according to decisions made by the governments of France, New Zealand, and the European Food Safety Authority. China has declared its intention to use all available technology, including cloning, to create a beef herd that is on pace with that of the United States in terms of quality.

Farmers and ranchers can increase the rate of reproduction of their most productive animals by cloning, which improves the production of safe and nutritious food. Since the healthiest animals are reproduced by cloning, fewer antibiotics, growth hormones, and other chemicals are used. Cloning will help consumers by producing safer, more consistent, and healthier meat and milk. It is possible to preserve endangered species through cloning. For instance, panda cells are held in reserve in China in case the extinction of this species poses a threat to its population. Genetic selection combined with artificial insemination has led to an increase in milk production of 100 pounds per cow annually in dairy cattle. The ability to clone genetically superior females intensifies genetic selection even as it may raise the degree of inbreeding Cloning animals is a dependable method of maintaining herds at the greatest possible quality and replicating superior livestock genetics.

7.5.8. TRANSGENIC ANIMALS

Transgenic livestock came into existence in 1985 with the production of the first transgenic pigs and sheep. Transgenic mice, rabbits, pigs, sheep, cows, chickens, and fish have been created by incubating sperm cells with foreign DNA and fertilizing them in vitro or in vivo. Transgenic mice were created at a rate of up to 4% when stem cells were transplanted into busulfan-treated recipient males. The most importance for generating transgenic farm animals to increase the growth rate and milk production. Transgenic technology advancements offer the chance to modify milk's composition or manufacture whole new proteins in milk. the first transgenic cow, Rosie, produced human alpha-lact-albumin-enriched milk at 2.4 g per liter. This transgenic milk is a more nutritionally balanced product than natural bovine milk and could be given to babies or the elderly with special nutritional or digestive needs [31, 32].

In an effort to separate valuable pharmaceutical proteins from milk, a number of private companies have created transgenic cows, sheep, goats, and pigs that express transgenic genes specifically targeting the mammary gland. Transgenic mice have been created that alter the physical and functional properties of mouse's milk protein system. Increased milk yield or composition can be advantageous for cattle, sheep, and goats raised for meat production. Heat-tolerant livestock breeds, like Bos Indicus cattle, are necessary for the increase of agricultural productivity in tropical areas. Bos Indicus cow breeds do not, however, yield a lot of milk. Weaning weights in Brazilian cattle of the Nelore or Guzerat breeds may be significantly increased by increasing milk production to just 2-4 litres per day. Dairy cows carrying these types of transgenes could become available by 2025. Using transgenic cows could result in the gradual separation of the genetic backgrounds of herds being used for fluid milk production from those used for producing milk for cheese manufacturing [31].

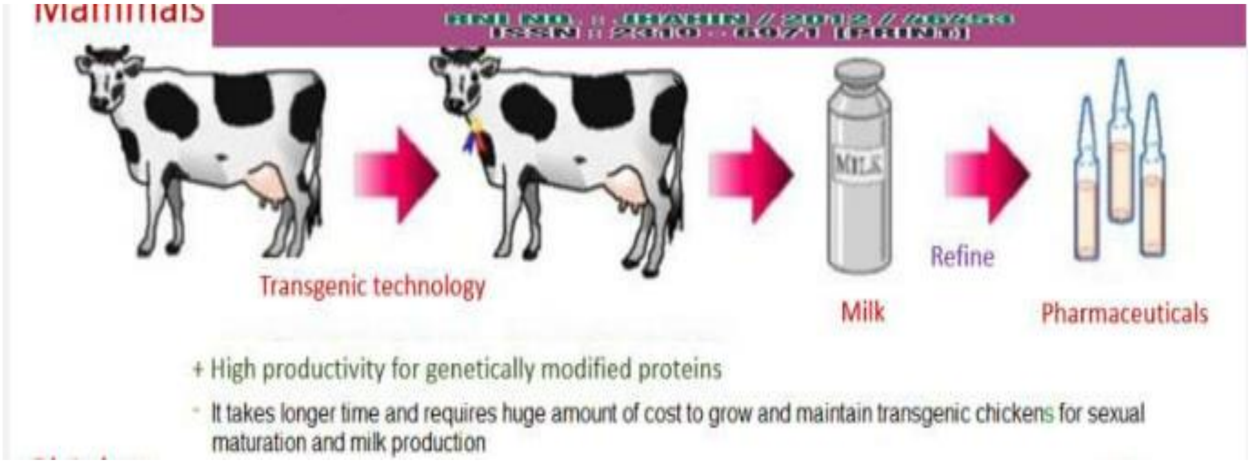

Figure 18: Advantage of transgenic technology

The transgenic sheep with wool keratin and keratin-associated protein (KAP) genes could be utilized to alter the protein composition of wool fibers, resulting in fiber types with improved processing and wearing qualities (Figure 18).

Aquaculture transgenic research is rapidly developing on a global basis. Fish and shellfish have a high fertility rate. Fertilization is frequently easy, and fertilized eggs develop outside the body, requiring no major manipulation, such as preimplantation. As a result, making transgenic fish or shellfish is rather straightforward. In Australia, the focus is on the possible use of transgenesis to control wild populations such as European carp [33].

The emergence of transgenic livestock technology revolutionizing the course of biological research offers a direct approach for improving the quality (e.g., milk quality) and efficiency (e.g., growth) of livestock production, developing disease-resistant livestock and for providing a system for generating pharmaceuticals or nutraceutical recombinant proteins and xenotransplantation products. In transgenic animal production, efficient gene delivery into target cells is critical. Pronuclear microinjection and somatic cell nuclear transfer are the most widely used transgene delivery methods for producing transgenic livestock [34].

1.6. Concluding Remarks

When it comes to raising domestic animals and feeding the world's expanding human population, biotechnology plays pivotal role. Reproduction and genetics are essential preconditions for a productive livestock operation. The expansion of our perspectives brought about by knowledge brings with it both opportunities and risks. Issues pertaining to genetic engineering and biotechnology in the production of food and medicine have been widely reported in recent times. Researchers have revealed how to use new biotechnology techniques to meet the nutritional needs of the world's growing population.

At this point, the most advanced form of biotechnology is the direct and artificial manipulation of domestic animal germplasm. Animal breeding is a particularly promising field for biotechnology applications. The field of animal breeding and genetic advancement has been, is, and will continue to be greatly impacted by biotechnology.

Author Contributions
All authors equally contributed for conceptualization, validation, writing original draft, review, editing and visualization.

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Conflicts of Interest
The authors declare no conflict of interest.

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