

RECENT ADVANCES IN DAIRY ANIMAL BREEDING AND REPRODUCTION: A REVIEW

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ARTICLE INFORMATION

Article History:

Received: 29th October 2022

Accepted: 15th December 2023

Published online: 31st December 2023

Author's contribution

MN article writing and editing, MD supervise and revise the manuscript.

Key words:

Animal breeding, Biotechnology, Dairy Innovation, Reproduction

ABSTRACT

This review has highlighted the transformative impact of recent technologies on dairy animal breeding and reproduction. Recent advances in the breeding and reproduction of dairy cows involve the integration of molecular genetics, cytogenetics, and reproductive biology into animal breeding practices. This integration of precision technologies has played a critical role in increasing the efficiency and productivity of breeding programs. The development of modern genetic technologies, such as genome mapping, marker-assisted selection, and transgenesis, has revolutionized the identification of superior genetic traits, contributing to accelerated genetic progress in dairy herds. Furthermore, advancements in reproductive technologies, including artificial insemination, embryo transfer, sperm sexing, and synchronization of estrus and ovulation in dairy cows, have supported the optimization of breeding strategies and facilitated improvements in economically important traits in livestock. Efforts have also been directed toward advancing the early detection of estrus stress in dairy cows using sensitive physiological indicators and sensor technologies, aiming to enhance decision-making in estrus management on dairy farms. In conclusion, recent improvements in the breeding and reproduction of dairy animals have demonstrated significant potential for enhancing reproductive efficiency, profitability, and the quality of milk and milk products. However, these technologies face limited applicability in developing countries due to challenges such as poor infrastructure, low costs, or a lack of human resources. Therefore, it is imperative to develop cost-effective technologies tailored to local and regional contexts, subsequently facilitating their broad dissemination within these regions.

1. INTRODUCTION

Global milk production is expected to rise by 1.6 percent per year, reaching 997 million tons in 2029 (FAO, 2021). In 2021, the total amount of milk produced by cows was 544 million metric tons (Shahbandeh, 2022); herd growth and yield growth contribute to the rise in milk production. Nonetheless, milk production in India, Pakistan, and several African countries that practice grazing-based animal husbandry has risen, mainly because of an increase in herd size rather than an increase in yield (Nimbalkar *et al.*, 2021). This plays a crucial role in mitigating food insecurity in various developing nations,

serving as a primary source of both income and sustenance for a significant proportion of the rural population (FAO, 2011). Through the development of dairy farming, better balanced rural economies are possible, as well as reducing poverty, unemployment, and income inequality (FAO, 2009). Moreover, milk produced from small- and large-scale farms contributes to every nation's economy at the micro-level and, consequently, the global economy at the macro level (Nimbalkar *et al.*, 2021). However, this contribution depends on the factors that determine milk production, including feed availability and quality, health, management, and the genetic makeup of the animal, which fits it to a specific production system and the application of new technologies (Kumar, 2017).

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The development and application of these new technologies in breeding and biological techniques designed to increase reproduction and production efficiency through automated heat detection, embryo transfer, superovulation, and synchronization techniques have had a significant impact on the contribution of dairy farming to global economic growth (FAO, 2021). However, these novel farm innovations are suitable only for a particular area, physiological stage of animals, and economically viable options to enhance animal productivity. Therefore, low-cost and user-friendly dairy farming innovations suitable for all kinds of farms, maintained under rural conditions existing in different tropical countries are in need to enhance animal productivity and henceforth farmers' socio-economic welfare. This review aims to provide new insights into the most recent research and technologies for improving dairy animal breeding and reproduction.

Overview of development of global milk production

The global dairy industry is experiencing massive transformation whereas addressing a number of challenges, favorable long-term consumer trends, and otherwise emerging technologies, present a number of opportunities. According to FAOSTAT (2018), more than 270 million dairy cows were inhabiting the world including dual-purpose and 2600 kg/cow/year were the global average milk yield. However, the average milk yield /cow/ year in most of the developing countries was below 6000 kg/cow/year with 87% of the cattle population. On the other hand, 33 countries with about 13 % of the global livestock population produce about 40% of the world's milk production. Ensuring food security through increasing animal productivity is the primary focus of the dairy sector (Clay *et al.*, 2020). This will be accomplished through continued selection strategies for milk yield, productivity, and functional traits (e.g. adaptation, welfare, resilience).

The differential selection process was undertaken around 10000 years ago starting from the early stage of cattle domestication and resulted in the development of nearly 1200 cattle breeds (FAO, 2015), with distinct characteristics such as milk yield, milk composition, environmental adaptation, coat color, body size, fertility, and overall resilience. Currently, only three breeds account for 95 percent of high-yielding dairy cows raised in the world's major dairy producing regions: Holstein (or Holstein-Friesian), Jersey, Brown Swiss, and their crosses. The global spread of these few breeds is primarily due to their higher levels of milk production and responsiveness to high-input production systems.

Generally, a continuously growing consumer demand for global milk and milk products, and technological advancement in dairy processing industries with advanced infrastructure to transport and store large amounts of dairy products were the main driven factors for the development of milk productivity which also compels dairy farmers to become more competitive. As a result, many industrialized or developed countries' overall milk yield has been raised, whereas in these countries, the overall number of dairy farms and cows has reduced, and larger herds are becoming less common (e.g., the United States and China; FAOSTAT, 2020).

Biotechnology Options for Improving Livestock Production

The traditional way of livestock productivity is no longer sustainable due to the dramatic growth of population growth and livestock product demand. A new insight that provides new opportunities to enhance livestock productivity in a way that alleviates poverty, improves food security and nutrition and promotes sustainable use of natural resources was mandatory. Therefore, scientists discover novel inventions to alleviate the above-said problems called biotechnology.

In recent years biotechnology is being used primarily for commercial and socioeconomic purposes to improve livestock production and productivity due to the fact that the livestock sector is a pillar of economic growth in developing countries. It creates an unpredicted horizon to improve the productivity of animals through increased growth, carcass quality and reproduction, improved nutrition, and feed utilization also, improved quality and safety of animal feed, improved health and welfare of animals, and reduced waste through more efficient utilization of resources (Roland, 2013).

Animal breeding and genetics

A significant and persistent genetic improvement has been made in livestock over the past several decades (Hill & Bunger 2004; Hill 2008). In recent years, the focus of animal breeding has rapidly shifted from short-term production goals to animal functionality, cost reduction, consumer perception, and product quality, resulting in overall animal production sustainability and long-term economic returns. Genetic selection for higher milk yield has been a key driver of dairy intensification, resulting in the development of highly specialized milk production systems with increasing herd size and a heavy reliance on cereals and protein sources (FAO, 2006).

Despite significant signs of productivity progress, the dairy industry's long-term success is dependent on the adoption of more sustainable breeding goals and management practices, particularly from an agroecological standpoint (Phocas *et al.*, 2016). Current

high-producing systems must be refined to place a greater emphasis on animal health and welfare, environmental efficiency, climatic adaptation, and increased preparedness for future challenges through the preservation of a diverse genetic pool. Several of these traits have recently been included in the breeding goals of some breeding programs, but there is still room for significant improvement.

Moreover, milk production and composition have been the primary selection goals in dairy cattle breeding programs for centuries (Miglior *et al.*, 2017), and as a result, milk yield has increased dramatically. Economically, the success of selection for improved milk yield or feed efficiency in high-producing dairy cows is primarily attributable to reduced maintenance requirements as production levels increase (Brito *et al.*, 2020a). The economic return from increased milk yield has been the primary driver of continued genetic selection for higher milk yield. In addition, increased milk yield is frequently regarded as a critical solution to the global challenges of ensuring food security and reducing greenhouse gas emissions, as dilution of maintenance results in both improved feed efficiency and lower methane emissions per kg of milk produced (Capper *et al.*, 2009).

Furthermore, temperament (and other behavioral traits), physical and anatomical variables (e.g., coat color, body size), and milk production have been the key traits under artificial selection (primarily based on phenotypic performance) since domestication. The array of traits targeted for improvement has expanded significantly over the last five to six decades as a response to the dynamic requirements of dairy producers, consumers, and society in general, thanks to methodological advances in the areas of quantitative genetics, animal breeding, and phonemics. As multi-trait selection became the norm in dairy breeding programs, the development of selection indexes became critical in balancing each individual's genetic merit for each trait under selection, based on economic value or desired genetic gains (Byrne *et al.*, 2016; Cole & Van Raden, 2018).

Animal breeding uses genetic marker technologies

Traditionally, animal breeding programs rely solely on phenotypes and pedigrees, whereas recent advances in molecular genetics and statistical methods for quantitative trait loci (QTL) mapping have made it possible to identify genetic factors that influence economically important traits. It is also important to identify genetic regions influencing phenotypic variation of complex traits through genetic interactions and environmental factors. These advancements have the potential to significantly increase the genetic improvement of livestock species through the use of MAS of specific loci, genomic

sequence selection, gene introgression, and positional cloning (Aguet *et al.*, 2023). The development and application of QTL mapping allow marker-assisted selection (MAS) (targeting specific loci for genetic improvement), genomic sequence selection (utilizing genomic information for trait enhancement), gene introgression (incorporating beneficial genes into breeding programs), and positional cloning (identifying and isolating genes of interest). The incorporation of detected QTL into genetic evaluation has the potential to improve selection accuracies, thereby expediting the genetic improvement of animal productivity (Jiang *et al.*, 2010).

Marker-assisted selections (MAS) and gene-assisted selections (GAS)

The process of genetic improvement involves selecting outstanding individuals from a population to produce higher yields in future generations. Long-term genetic evaluations were used by dairy animal breeders to identify superior animals. The use of these animals improved phenotypic measures for milk production and milk components, especially in Holstein cattle. However, there are some drawbacks to choosing based on predicted breeding values. This method of selection has a limited ability to improve low-heritable traits without negatively affecting production. These traits are frequently associated with disease resistance, reproduction, productive life duration, and some conformational traits associated with fitness (Sonstegard *et al.*, 2001).

Moreover, the evolution of intensive dairy systems has been fueled by a steady stream of innovations and technological breakthroughs, with conventional genetic selection playing a significant role in recent decades (Miglior *et al.*, 2017). Animal breeding and genetics have been extensively conceptualized in artificial and standardized environments, where the linear equation: P (observed phenotype/performance) = G (additive genetic merit) + E (environmental effects) proved to be extremely efficient, particularly under controlled environmental conditions and high-input production systems.

In addition to genetic selection, the dairy industry has benefited from significant advances in nutritional practices, precision management, widespread adoption of reproductive technologies (e.g., artificial insemination, embryo transfer, sexed sperm), and precision health care and management. There is no doubt that many of these advancements have enhanced the effectiveness of genetic selection to increase productivity. Therefore, selecting a high-producing dairy cow is more than just the result of high genetic merit for key biological mechanisms and appropriate environmental factors; it also reflects complex positive feedback between these two

components that occurred during the industrialization and intensification of dairy production.

Transgenes of Dairy animals

There are several applications for producing transgenic farm animals with exogenous DNA stably incorporated into their genome so that the 'transgene' is transmitted to offspring in a Mendelian fashion. In addition to the obvious scientific interest in studying genes and their regulation, transgenic animal technologies have been proposed to accelerate livestock improvement by introducing new genes or modifying the expression of endogenous genes that regulate economically important traits such as milk production traits (Wheeler, 2003). The ability to insert genes into livestock embryos, incorporate those genes, and ensure their stable transmission into the genome of the offspring will allow major genetic advances in animal agriculture to be realized. Genes that increase productivity (milk yield) or reduce costs (disease resistance) are most likely to be found in the species in question. If a gene is well characterized enough to be used in transgenesis, it will also be possible to genetically characterize individuals carrying the gene, making direct selection and propagation highly efficient. The majority of focus in dairy animals has been on genes that alter fat or protein synthesis in the mammary gland (Naqvi, 2007). This technology has been utilized to produce transgenic goats for the production of recombinant human protein therapeutics in their milk, achieved by introducing and stably integrating an engineered piece of DNA into the animal's genome, thereby directing the expression of the recombinant protein in the milk during the lactation period (Gavin *et al.*, 2018).

Furthermore, research has focused on the development of a universal gene knock-in strategy for mammary gland-specific expression of recombinant proteins in dairy cattle, aiming to overcome the limitations of random transgene integration and subsequent epigenetic silencing (Lee *et al.*, 2014). Additionally, transgenic goats have been engineered for the production of biosimilar antibodies in their milk, demonstrating the potential for using transgenic dairy animals as a production platform for biopharmaceuticals (Laible *et al.*, 2013). These applications highlight the diverse uses of transgenic technology in dairy animals, ranging from the modification of milk composition to the production of valuable recombinant proteins.

Progeny testing

The practical and best techniques, the performance of sires are evaluated based on their daughters' performance. AI services are delivered in a certain place for a particular breed in its native tract, and progeny produced in this way is evaluated for their performance. Progeny testing is practical and the best option for achieving genetic

improvement in that breed (Vidya Nimbalkar *et al.*, 2021). The effectiveness of progeny testing has been enhanced through the incorporation of genomic pre-selection (GS-PT), which allows for the selection of sires based on their estimated breeding values (EBV) and the performance of their progeny (Yamazaki *et al.*, 2014).

The advantages of GS-PT over traditional progeny testing (PT) include increased annual genetic gain and more accurate selection of sires with higher heritability (Yamazaki *et al.*, 2014). Some benefits of progeny testing in dairy animals include:

1. Identifying superior genetics: Progeny testing helps identify bulls with high genetic merit for various traits, such as milk production, quality, and health.
2. Genetic improvement: The evaluation of bulls through progeny testing has been a major source of genetic improvement in dairy animals.
3. Large-scale implementation: Progeny testing programs can be extended to farmers' herds, where large numbers of daughters per bull can be produced and recorded, increasing the accuracy of the selection process (Das *et al.*, 2014).
4. Combining genomic pre-selection with progeny testing: The incorporation of genomic pre-selection into dairy cattle progeny testing has shown to increase annual genetic gain compared to traditional progeny testing (Yamazaki *et al.*, 2014).

Overall, progeny testing in dairy animals is a valuable tool for evaluating and selecting superior genetics, leading to improvements in the performance and traits of dairy animals. The integration of genomic pre-selection and progeny testing has further enhanced the effectiveness of this method, allowing for more accurate and efficient selection of sires with high genetic merit.

Molecular marker-assisted introgression (MAI)

Molecular marker-assisted introgression (MAI) is a breeding technique that utilizes molecular markers to identify and select desirable traits in dairy animals. This technique involves the identification of molecular markers linked to specific traits of interest, such as milk production or disease resistance, and the use of these markers to select animals with the desired traits for breeding (Hariyono *et al.*, 2022). Those markers are used to help livestock breeders choose individuals who express the introgressed gene. In conventional breeding, a series of backcrosses to the recipient parent is usually performed. However, the use of molecular markers shortens the time and cost for used backcrossing cycles required to select and identify the desired individual. It is also increasing the efficiency of breeding programs to improve livestock traits such as growth, meat quality,

wool quality, milk production and quality, and disease resistance (OMIA, nd).

Additionally, MAI has been used to introgress desirable traits from wild or exotic breeds into domesticated dairy animals, resulting in improved performance and adaptation to changing environmental conditions for example, the use of molecular markers has been shown to be effective in selecting thermo-tolerant animals in dairy cattle breeding, allowing for the selection of superior thermo-tolerant animals through marker-assisted selection (MAS) (Hariyono *et al.*, 2022).

In general, the use of molecular marker-assisted introgression in dairy animal breeding has shown promise in improving the efficiency and effectiveness of breeding programs, leading to improved performance and adaptation to changing environmental conditions.

Screening for undesirable genes

Screening for undesirable genes in dairy animals is essential for maintaining the health and performance of the herd. Some examples of undesirable genes in dairy animals include those associated with genetic disorders, such as Deficiency of Uridine Monophosphate Synthase (DUMPS), Factor XI deficiency (FXI), and Complex Vertebral Malformation (CVM) (Ghanem & Nishibori, 2008). These genetic disorders can lead to poor production and reproduction performance, structural unsoundness, and other health issues in dairy animals (Magotra *et al.*, 2020). Molecular markers can be used to trace and document genetic diseases and physical defects in livestock animals. The genetic changes and DNA mutations that manifest in protein structure and function are easily traced as the cause and origin of these problems (Womack, 2005).

To screen for undesirable genes in dairy animals, the following methods and techniques can be employed:

- **Genetic testing:** Advanced genetic testing technologies, such as polymerase chain reaction (PCR) and DNA sequencing, can be used to detect the presence of specific mutations or genetic markers associated with undesirable traits.
- **Phenotypic assessment:** Visual observation and evaluation of the animal's physical appearance, behavior, and performance can help identify signs of genetic disorders or other undesirable traits.
- **Pedigree analysis:** Analyzing the pedigree of animals can help identify potential carriers of undesirable genes, as well as plan for future breeding strategies to minimize the risk of passing on these traits to offspring.

- **Progeny testing:** As mentioned earlier, progeny testing can be used to evaluate the genetic potential of animals, particularly bulls, for their performance and traits. By evaluating the offspring's performance, the bull's genetic merit can be assessed, and the presence of undesirable genes can be identified (Magotra *et al.*, 2020).

Then after, animals carrying defective genes are easily identified using DNA testing and are culled from the livestock breeding program.

Generally, intensive selection for production traits, combined with the intensification of dairy cattle production systems, has resulted in animals that are more susceptible to behavioral, physiological, and immunological disorders. The discovery of precise technologies to measure novel traits like resilience, welfare, and environmental efficiency used across multiple production systems has a significant impact.

Advancement in dairy animal Reproduction

The goals of using reproductive biotechnologies in livestock are to increase output, improve reproductive efficiency, and genetic improvement rates. Many options for managing the reproduction of small and large ruminants have become available over the years (Said *et al.*, 2020). The main technologies that are widely used are artificial insemination (AI) and sperm preservation. Assessing sperm fertilization capacity, sexing sperm, synchronization and fixed-time insemination, superovulation, embryo transfer (ET), and in vitro embryo production (IVEP) are other techniques that can improve reproductive and pregnancy rates. Molecular DNA markers can also be used to improve genetics through marker-assisted selection (MAS), as well as to characterize and conserve an animal's genetic resources.

Estrus synchronization

Estrus synchronization is a technique by which most of the females in a population or a herd can be brought into estrus at a predetermined time. Recent advancements in estrus synchronization in dairy animals have improved the efficiency and effectiveness of breeding programs. These programs have become standard components in the current breeding management of cows in most dairy industries, and many are based on protocols that allow timed inseminations (TAI) to overcome the practical difficulties associated with estrus detection (Channo *et al.*, 2021; Macmillan, 2010). These difficulties are exacerbated in modern herds of high-producing cows due to increasing herd size, individual animal monitoring difficulties, and often subjective observations (Macmillan, 2010).

The major limitation of estrus-synchronization programs is their inability to induce potentially fertile estrus and ovulation in non-cycling cattle (i.e., prepubertal heifers and anoestrous suckling cattle). However, the most recently developed programs include protocols for resynchronization following first or subsequent inseminations, which may involve selected forms of hormonal intervention during the diestrus and pro-estrous periods following TAI or following pregnancy diagnosis by ultrasound from 28 days after TAI (Macmillan, 2010). PGF2 α , GnRH, and Progestin are the most important Hormones for estrus synchronization. Heifers respond extremely well to the MGA/PGF2 α system (Amare & Ayalew 2021).

Overall, recent advancements in estrus synchronization have improved the accuracy and efficiency of breeding programs, leading to improved reproductive efficiency and genetic selection in dairy animals. It also provides significant economic benefits for dairy farmers and breeders through reduced labor costs, improved pregnancy rates, cost savings on feed and housing, and increased profitability.

Artificial insemination (AI)

Artificial insemination is the most widely used biotechnology in livestock, particularly in cattle production to deposit a proven sire's stored sperm directly into a cow's uterus (Vidya Nimbalkar *et al.*, 2021). It is still considered to be one of the most important assisted reproductive technologies (Jacquelyn & Laura, 2008). The technique is used to improve desired characteristics quickly through intensive genetic selection. This innovative technique has achieved benefits such as facilitating the use of superior quality semen without the expense and risk of sire ownership; and lowering the risk of introducing venereal diseases into the herd (Vidya Nimbalkar *et al.*, 2021). It also eliminates the need to keep a bull for natural service and aids in the utilization of excellent germplasm up to the fullest extent. Because AI is simple, inexpensive, the quickest, and most effective method of breeding, developing countries such as India could become the world's top milk-producing country. Moreover, about 90% of genetic improvement in a commercial herd is dependent on AI. Furthermore, the sperm from the superior sire can be preserved for a long period by the technique of sperm cryopreservation in liquid nitrogen temperatures at -196°C. However, the viability of sperm is critical to the success of AI (Jindal & Sharma 2010).

Embryo transfer and multiple ovulations (MOET)

Embryo transfer technology (ETT) is one of the most recent tools available for faster livestock improvement worldwide, particularly for exploiting the genetic potential of high-quality females and males at the same

time (Said *et al.*, 2020). It is the process of implanting embryos in the uterus of a female (cow) to establish a pregnancy. The process involves three steps: superovulation with follicle-stimulating hormones, embryo collection surgically or nonsurgical, and embryo transfer. Prior to the development of this technology, a superior/high milk-producing cow could only produce a limited number of offspring in her lifetime. Higher technology costs and a low conception rate may be factors limiting its implementation (Vidya Nimbalkar *et al.*, 2021). The benefits of embryo transfer include breed preservation and conservation, disease-free herd creation, economical livestock transport, and rapid multiplication of elite female breeding stock.

Furthermore, various hormone protocols are being used to achieve group calving or desired calving in a year. MOET has the potential for genetic improvement by increasing the selection intensity on the female by increasing the number of calves, either male or female, from genetically superior donors. The application of MOET can also lead to increased reproductive capability and ability of precious or valuable animals, as well as an increase in the percentage of genetic improvements in the herd (Faizah *et al.*, 2018). It can also be used to screen bulls for inherited defects.

Embryo splitting

Embryo splitting is the artificial microsurgical splitting of an embryo which results in the formation of twins or multiples. It is used to increase the rate of genetic improvement in dairy cattle through embryo transfer and splitting. The genetically identical embryos can continue to develop after separation. Before embryos are transferred to a surrogate female, the morula or blastocyst stages can be cut into two equal halves using an inverted microscope connected to a micromanipulator and a microsurgical knife. This method can produce genetically identical animals. This procedure appears to mimic the natural process of producing monozygotic twins (Said *et al.*, 2020). Embryo splitting is used to increase the rate of genetic improvement in dairy cattle through embryo transfer and splitting.

In general, the use of embryo splitting, and transfer has been integrated into dairy cattle improvement programs, offering advantages such as increased genetic gains and greater control over recording, breeding, and selection, leading to a larger proportion of the possible genetic gains being realized in practice. Additionally, embryo manipulation and gene transfer technologies have had a major impact on genetic strategies in animal production, allowing for advancements such as embryo splitting producing monozygotic twins, in vitro fertilization, and chimera production of tetra parental animals. The importance of embryo splitting in dairy animal breeding

lies in its potential to increase the rates of genetic improvement, enhance control over genetic strategies, and facilitate the production of transgenic animals for the purpose of generating valuable proteins in the milk of dairy animals.

Sperm sexing

In dairy farming farmer's desire is to get female calves, sperm sexing is a guarantee for them. The 'Y' chromosomes in sperm cells are removed through the sorting process; sexed semen predominant with 'X' chromosomes can ensure the birth of a female calf. The application of this technology is popular among dairy farmers are a reduction in economic burden and the production of a greater number of female calves as future productive cattle. There have been advancements in the development of cost-effective indigenous sperm sexing techniques, such as microfluidics and bio electromechanical systems (BEMS), which may hold the key to the development of a portable device for semen sexing with minimal tampering of the sperm structure (Rai, 2018) (Figur 1). Although this technology has been used on a variety of species, the vast majority of pregnancies have been in cattle, almost entirely as a result of sexed sperm that has been frozen (Seidel, 2007).

The use of sperm sexing in dairy animal breeding offers several advantages this includes.

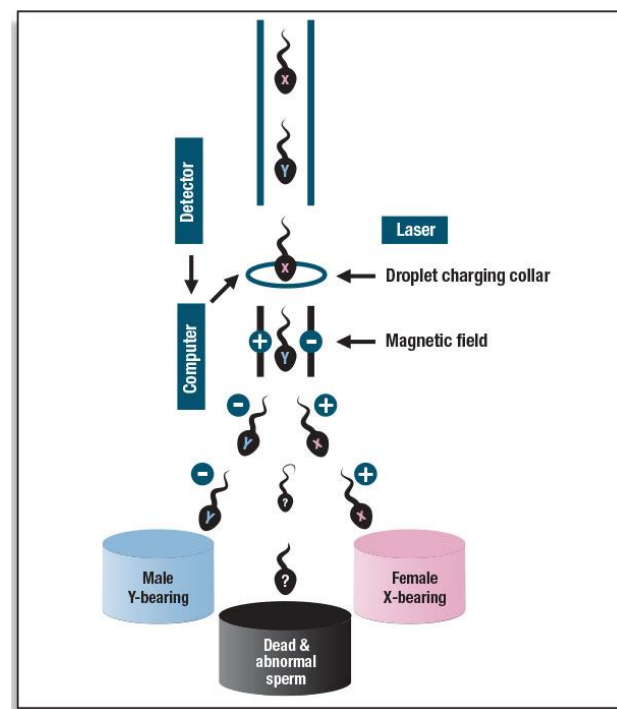
1. Improved productivity: By producing more female dairy animals, sperm sexing can help increase the productivity of dairy herds, leading to higher milk yields and better economic returns for dairy farmers.
2. Cost-effective approach: Sperm sexing is a cost-effective approach for achieving sex pre-selection, as it can lead to more female dairy animals, which are more valuable for dairy farmers, while male dairy crossbred animals are more valuable for beef production.
3. Genetic improvement: Sperm sexing can help improve the genetic makeup of dairy herds by focusing on the production of female dairy animals, which are more valuable for dairy farmers.
4. Prevention of genetic disorders: Pre-implantation sexing of embryos can help diagnose genetic disorders at the prenatal stage, benefiting both the management, production, and breeding programs of livestock and the diagnosis of genetic disorders.

Although the evolution of sexed semen in dairy animal breeding has been marked by significant technological advancements and a growing recognition of its economic and practical applications, Vidya Nimbalkar *et al.* (2021) states that the higher cost of semen, combined with the low conception rate, are important factors to consider before using it, especially in heifers or primiparous

animals. In addition, the technique does not involve any genetic modification or manipulation, and therefore, it does not have a significant impact on genetic diversity.

In general, it enables the production of more female dairy animals, leading to increased productivity and better economic returns for dairy farmers and breeders.

Figure 1 Semen sorting process



Embryonic stem cells

Embryonic stem cells (ESCs) are stem cells derived from the donor mother animal's undifferentiated inner cell mass of an embryo. Stem cells are pluripotent cells that can self-replicate and develop into specialized cells. It can be found at various stages of fetal development and in a variety of adult tissues. In the laboratory, stem cells are manipulated to accept new genes, which can then change their behavior. This procedure entails removing the donor mother's ovaries and dosing her with progesterone, which alters the hormone environment and allows the embryos to remain free in the uterus. The embryos are harvested after 4–6 days of intrauterine culture and grown in vitro until the inner cell mass forms egg cylinder-like structures (Said *et al.*, 2020). Moreover, the use of embryonic stem cells allows for the direct manipulation of the germline, making it possible to add new milk-protein genes to dairy animals and remove or replace endogenous genes (John & Clark, 2005).

Recent advancements in transgenic technology have made it possible to produce transgenic large domestic animals, such as sheep and cattle, through nuclear

transfer, which opens up a means of ruminant transgenic production with an efficiency that entitles us to consider it a serious alternative to microinjection. Additionally, the isolation and culture of spermatogonial stem cells (SSCs) from cryopreserved dairy goat testicular tissues have been explored as an efficient procedure for cryopreserving dairy goat testis tissue, offering an efficient way to preserve SSCs for infertility and rare animals and eminent livestock (Ze-hua, 2011). The use of embryonic stem cells in dairy animal breeding has shown promise in enhancing milk protein properties and improving genetic selection, leading to improved productivity and economic returns for dairy farmers and breeders.

In general, improvements in reproductive performance had the largest influence on revenues followed by energy efficiency and then by disease reduction. The adoption of reproductive technologies has a great impact on the incidence of ketosis, milk fever, conception rates at first service, metritis on days open, unrealized milk, veterinary costs, labor, and discarded milk.

Application of biotech in Ethiopia

Ethiopia has an abundance of fauna and flora genetic diversity, but its biotechnology facilities lack precise information about their capabilities, capacities, and associated technical and administrative gaps. In most cases, biotech equipment fails to operate due to either a lack of skilled technicians or an inability to maintain it. In light of the foregoing, the Ethiopian government has designated biotechnology as a critical science that requires special attention and support to sustain the country's rapid economic growth. Biotechnology is used as critical support for GT-I and II, to drive economic and social development and achieve middle-income status by 2025 (Abu & Rachel, 2018).

Moreover, information from different literature shows that seven institutions with a various developmental stage, primarily tissue culture, but also bio-fertilizers, molecular markers, embryo transfer, immunology, vaccine, diagnostic kit development, and epidemiology. Ten centers have laboratories that range from basic to well-equipped, and a few more are in the process of being built. In general, the future success of biotechnological research and development in Ethiopia is dependent on the government's attention to capacity building and the level of collaboration among institutions (Dereje, 2011). To exploit the indigenous livestock's genetic potential the Ethiopian government planned and implement the following applied research.

- Detection of selected signatures for trypano-tolerance traits/genes in Ethiopia cattle.

- Genome-wide association studies for egg production, egg quality, and natural antibody (NAB) traits in indigenous chicken in Ethiopia.
- Harnessing fecundity and muscle growth gene to improve the productivity of indigenous sheep in Ethiopia using a genomic approach.
- Other related animal biotechnology research includes the development of vaccines and diagnostic kits (Abu & Rachel, 2018).

2. CONCLUSION

The demand for milk and products dramatically increased as a result of rapid population growth and income growth, however, the resources are limited, and building sustainable food production is a global challenge. The application of biotechnology in the dairy sector plays a significant role in solving problems in the areas of breeding, reproduction, management, diseases and milking to meet the growing demand for milk and products. The use of genomic selection techniques has proven to be a major breakthrough, enabling accurate prediction of genetic performance at early life stages. This not only accelerates the pace of genetic improvement, but also minimizes the generation interval, accelerating the overall progress of dairy herds. Moreover, advanced reproductive technologies such as sexed semen and in vitro fertilization have given dairy farmers greater control over breeding, thereby optimizing the production of desirable traits. It has been proven that the integration of these cutting-edge technologies in breeding and reproduction has led to significant improvements in the precision and efficiency of breeding programs, which in turn have led to sustainable and economically viable strategies.

Even though recent advances in dairy cow breeding and reproduction have introduced a new era in dairy farming, enabling higher productivity and genetic advantages for dairy farmers, these advanced technologies have been primarily used in developed countries such as the United States, Brazil, Europe, and others due to a lack of infrastructure, technical and educational capacity, and economic factors. Therefore, there is a need to develop local and region-specific technologies and disseminate them in similar socio-geographical regions to build sustainable dairy sectors in these nations. In addition, the following issues should be addressed to improve the dairy sector in developing countries.

1. Government intervention provided a pillow for rather soft budgeting of farms with dairy production,
2. The mobilization of research, knowledge transfer and innovation became one of the priority areas for government organizations, research centers, NGOs,

and Universities in developing countries like Ethiopia.

3. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

4. DEDICATION

This paper is dedicated to the late researcher and advisor, Dr. Moges Derege.

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