ANIMAL BIOTECHNOLOGY

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Website: www.booksarcade.co.in

Year of Publication 2024

International Standard Book Number-13: 978-81-19923-16-8

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CHAPTER 1

INTRODUCTION TO ANIMAL CELL AND TISSUE CULTURE

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ABSTRACT:

Cell culture technology is a possible way of growing cells that involves using different areas of study. Modern biotechnology has seen the development of culture media, facilities for growing animal tissue, and tools for studying cells. These advancements are especially important for improving human health. This chapter talks about the basics of growing animal cells in a lab. It also looks at the history of this practice, different types of cultures, how to take care of them, and the tools used to study them. It also has labs for growing animal cells and safety rules for working with mammal cells in a controlled environment. In the animal tissue lab, it's really hard to stop germs from getting in. This chapter talks about how to stop germs from getting into the lab.

KEYWORDS:

Cell culture, Cell line, Genetic Engineering, Human Genome, Tissue Culture.

INTRODUCTION

Animal Cell Science studies the basic principles that underpin the productivity of animal cells in culture. Cell culture is no longer seen as a method for producing cell lines, but rather as an essential science for understanding the many cell-signaling systems, or switches inside cells that govern growth via gene expression regulation. Simply said, it is the fundamental need for comprehending a cell's intercellular and intracellular activity. The cultivation of animal cells is critical to the biotechnology sector. With recent developments in research into altering the environment to boost cellular growth in vitro, innovative chemicals for therapeutic and preventive usage have been developed. Animal cell culture techniques have been devised to produce the same medicinal compounds in big quantities. Prokaryotic cells have been genetically modified to create therapeutically significant chemicals[1], [2].

These recombinant compounds do not provide the anticipated outcomes in eukaryotic systems because to variations in glycosylation. Eukaryotic cells are favored for producing compounds and evaluating their toxicity and effectiveness. Tissue culture technologies have been developed in numerous biomedical applications to replace animal experiments. In vitro pharmaco-toxicology is currently a well-established science for investigating pharmacological activity and developing novel ways of treatment. Currently, cell culture techniques are employed for histotypic cultures that result in organ development. Hybridoma technique enables the bulk synthesis of monoclonal antibodies from cell culture medium. Viral vaccines may be created by mass culturing host cells. Recombinant DNA technique use cultivated cells as a source of mRNA, gene sequence, or rDNA expression vector. Cell culture systems may now be effectively employed in combination with transfection methods to investigate gene function[3], [4]. These systems contribute to the evolution of mechanisms that regulate cell development and differentiation, which may have a significant influence on

our understanding of human and animal illnesses. Tissue culture is the process of growing tissues or cells apart from the organism. This is often accomplished by the use of a liquid, semi-solid, or solid growth medium, such as broth or agar. Tissue culture is often used to describe the cultivation of animal cells and tissues. The primary goal of cell, tissue, and organ culture is to separate sections of the organism for research in experimentally controlled conditions. Intact creatures have a high degree of connectivity and interaction among their component components. Cultivation in vitro isolates cells from the influence of the organism as a whole and the products of all cells other than those injected into the culture. Artificial habitats may be built to mimic the natural physiological environment, or they can be changed at whim by intentionally introducing certain variables and pressures. Culture allows for the study of almost any form of cell or cell aggregation. Cine photomicrography may be used to investigate living cells, as well as direct, phase-contrast, interference, fluorescence, and ultraviolet microscopy. Fixed cells from culture are excellent for cytological, cytochemical, histological, histochemical, and electron microscopical examinations[5], [6]. Nutritional, biochemical, and immunological studies employ cell populations from monolayers or suspension cultures.

Organ culture, or the cultivation of complete organs or sections of them, is especially useful for studying development, inductive interactions, and the impact of chemical and physical agents on the physiological functioning of specific organs. Both cell and organ culture have uses in pathology, such as comparative, developmental, and diagnostic studies of tissues from healthy and sick donors, as well as research into carcinogenesis, somatic cell genetic diversity, virus susceptibility, and other topics. Cell cultures are commonly employed in microbiological studies, radiation impacts research, and medication screening, particularly for carcinogenic, mutagenic, and radiomimetic compounds. Organ culture is a development of tissue culture research methodologies; organ culture is capable of correctly modeling the functioning of an organ in different stages and situations using the real in vitro organ. Parts of an organ or the whole organ may be cultivated in vitro. The primary goal is to preserve the tissue's architecture and guide it toward normal development. This approach requires that the tissue never be disturbed or harmed. It consequently needs cautious handling. Growing organ cultures often utilize the same medium as tissue cultures[7], [8]. Organ culture methods are divided into two types: solid media and liquid medium.

The tissues acquired are adequate for short-term investigations. The cost and hassle of keeping inventories of established cell lines are eliminated. They are suitable for vaccine manufacture since the risk of in vitro cell transition to malignancy is reduced. Survival in conditioned media is as simple as it is difficult. However, there are some downsides. They are heterogeneous since several cell types are present. They also expect regular animal sacrifices. They are readily infected with latent viruses. Long-term studies cannot be conducted since they have a limited lifetime. Primary cell lines continue to divide at a rapid pace for an extended period and are readily passed on. Most primary cells cease multiplying after a high number of passes. Some cell lines can be passaged indefinitely in vitro. These are then referred to as established cell lines. In some circumstances, the shift from primary to established cell lines is smooth and gradual; however, in others, cell transformation or cell modification may produce this change. The converted cell grows quickly and may rapidly outweigh the other cell population, becoming the dominant cell type in the culture. Primary cell lines have the typical number of chromosomes, while established cell lines contain an unusual number of chromosomes. They have short doubling periods and are always aneuploid. They have the same needs regardless of where they come from. There is no evidence for spatial orientation.

They reach greater densities than primary cultures. They may spread from a single cell or as a dilute inoculum. In contrast to primary cultures, they may be established in suspension if necessary. A considerable number of established cell lines have been obtained without any prior exposure to a transforming agent. Such lines include the RMP promegakaryocyte line produced from rat bone that preserves multiple differentiated characteristics including the capacity to manufacture factor VIII, antigen, and fibrinogen. The fibroblast cell line 3T3-L1 is produced from mouse embryos and may accumulate lipids. However, it is uncommon to isolate cell lines spontaneously, and the ones that have been acquired are mostly from embryonic rodent tissues. Methylcholanthrene has been used extensively to create several cell lines, including the mouse L-cell line and the rat muscle line L6. Another carcinogen, azoxy methane, has been utilized to convert normal human colon mucosal cells into malignant lines with altered morphology, culture lifespan, soft agar growth, substrate adherence, and peanut agglutinin binding. Human mammary epithelial cells exposed to benzopyrene have a longer lifetime, and seemingly immortal cell lines may be obtained. These lines do not seem to be malignantly altered since they do not produce tumors in nude mice and exhibit little or no anchorage-independent growth[9], [10]. They do, however, seem more like tumor-derived mammary epithelial cells.

Biological pollutants include bacteria, molds, viruses, mycoplasmas, yeast, a few protozoans, and invertebrates. Cross-contamination from other cells in the culture might also be a deterrent. These biological contaminants might have come from a variety of sources, including contact with non-sterile equipment, media, solutions, or the touch of your things and clothing. Particulate or aerosol fallout during testing, manipulation of flasks, plates of culture vessels, transit from one location to another, or neglect while preparing for incubation all contribute to the problems. Accidents and errors can occur, typically as a result of worker negligence, and should be prevented at all costs. Bacteria, fungi, and yeast are widespread and have the potential to swiftly colonize and thrive in the rich nutritional environment supplied by cell culture, making them a persistent source of contamination.

In the absence of antibiotics, bacteria may be discovered in a culture within a few hours or days using direct microscopic inspection or medium turbidity measurement. Occasionally, a pH change is seen, followed by a floating cell population caused mostly by cell death. However, the use of antibiotics regularly causes microorganisms to become resistant, resulting in a latent infection that may be difficult to detect under the microscope. Due to their tiny size, viruses are tough to see and hence difficult to eliminate from media, solutions, or any other biological fluids. Viral infection of cells is not the primary worry; nonetheless, the possible health risks to laboratory staff are concerning[11]. Thus, extreme vigilance should always be used. When dealing with tissues or cells from humans or other primates, it is critical to prevent any transmission of viral infection (HIV, HBV, EBV, etc.). Protozoans, which include both parasitic and free-living amoeba, are mostly water-borne.

Protozoans induce damage similar to viral damage and may fully kill the culture within two weeks. Due to their sluggish development and physical similarity to grown cells, amoebas are difficult to identify in culture. Although such pollutants are uncommon, workers must be aware of the likelihood of their presence. Insects and arachnids are never the direct source of any contamination in cell culture. However, the presence of insects in and around lab spaces, particularly cockroaches, ants, flies, and mites, may cause contamination, including microbiological contamination. Robinson and his colleagues discovered mycoplasma in cell culture for the first time in 1956. Mycoplasmas typically infect at least 15% of cell cultures. Mycoplasmas are not harmless culture pollutants since they may affect cell function, growth, metabolism, and morphology, and cause chromosomal abnormalities and damage, as well as

plaque formation. Mycoplasma seems to be the smallest self-replicating creature; they lack a cell wall and have extremely strict and demanding development requirements, allowing them to thrive at large densities in cell culture with no evident evidence of contamination. Viruses are also tiny in size, capable of infecting 100% of the cells in culture, and cannot be removed from sera with membrane filtering. As a result, they have the power to affect every aspect of cell function, making viruses and mycoplasma the most dangerous, widespread, and disturbing culture contaminants. Some researchers have even dubbed Mycoplasmas the "crabgrass" of the cell.

When punch biopsies are conducted from human samples, the cultures that result are called explant cultures. These are broken into little pieces and placed in a Petri dish with sterile fluid for a few hours to enable cells to bind to the substrate. It might take days or even weeks to build a culture. It is the slowest approach available. The replicative capability of cultivated cells varies by cell type and species. Many cells may be passaged several times;however, some perish at an early stage. In rodent tissue, cells seldom divide forever. Cells from human tissue never form continuous cell lines unless they are treated with certain chemicals. Chicken cells are tough to sustain after a few doublings. In 1961, Hayflick and Moorhead investigated the ability of human fetal lung fibroblasts to divide in culture by counting the cells at each passage beginning with an explant from human tissue. They discovered a modest rise in growth rate (phase I). During this phase, some cells die and others develop. If the media is constantly fed, they grow at a consistent pace for an average of 50 generations (phase II), at which point the growth rate slows. The subsequent phase of accelerated cell death (phase III) leads to the full demise of the culture. Human cells can go through 20-80 passages in total;however, some may be shorter. The restricted replicative potential of human cells in culture is also known as the Hayflick effect, after its discoverer. Experiments on adult lung fibroblasts done in 1970 clearly show that the number of population doublings accomplished in the culture varies with donor age. The cells' lifetime increased linearly with the donor's age.

DISCUSSION

Biotechnology is using man-made ways to change the genes of living things to make new substances or do new tasks. Biotechnology has been used to make livestock and crops better since farming started by choosing the best ones to breed. Ever since scientists found out how DNA is put together in 1953, and especially after they figured out how to change it in 1970, biotechnology has been very important for changing the DNA of living things at the smallest level. This technology is used in medicine to make vaccines and antibiotics, and in farming to change the genes of plants. Biotechnology is used in industries like fermentation and cleaning up oil spills. It's also used in household products like laundry detergent. Animal biotechnology is when scientists and engineers use their knowledge to change living things for the better.

Animal biotechnology is used to make things, make animals better, and develop small organisms for farming. Making transgenic animals is the best example of using technology with animals. The simple method is based on nucleic acids, which are large molecules made of sugar, phosphate, and a nitrogenous base[12], [13]. The phosphate groups on these molecules are negatively charged. The complete set of DNA in the nucleus of Eukaryotic organisms is called the Genome. DNA is made up of two strands that stick together because of some special bonds. RNA molecules in eukaryotic cells move out of the nucleus, unlike DNA. Messenger RNA (mRNA) is studied a lot because it shows which genes are making proteins in the cell. In this section, you will learn about animal biotechnology, how genetic engineering works, and how it is used in animals.

Overview of Animal Biotechnology

Biotechnology, also known as biotech, is a field of science that uses living things like plants or animals to create useful products. It can also be any technology that uses living things to make or change products for a specific purpose. Depending on the tools and apps, it's often similar to other areas like biology, engineering, and manufacturing. Biotechnology is a type of technology that uses living things to make new products. Making beer and baking bread are examples of processes that are part of biotechnology. They use yeast to create the final product. Traditional methods use natural living things or ones that have been developed through breeding. Modern biotechnology often involves more advanced changes to the biological system or organism. Biotechnology is a technology that relies on biology. Biotechnology uses cells and molecules to make products that help us live better and stay healthy. Microorganisms have been used for a long time to make food like Bread and Cheese. They are also used to keep dairy products from going bad. Modern biotechnology gives us new and advanced products and technologies to fight serious and rare illnesses, protect the environment, feed people who are hungry, and use less and cleaner energy.

It also helps to make industrial processes safer, cleaner, and more efficient. Right now, there are over 250 Biotechnology Health Care Products and Vaccines that patients can use. They are mostly for diseases that couldn't be treated before. Over 133 million farmers all over the world are using technology to help them grow more crops, protect their plants from pests, and lessen the harm that farming does to the environment. The new developments in biotechnology are helping us prepare for and solve society's biggest problems. The word "biotechnology" was made up by a Hungarian engineer named Karl Ereky in 1917. He used it to talk about a way to make a lot of pigs all at once. He says that all jobs are biotechnology because they use living organisms to make things from raw materials. At the end of the 20th century, biotechnology became a new way of combining biology with technology. This subject did not grow quickly, but people found some ways to study it many years ago. In simple terms, biotechnology is about using living things to make helpful things. Biotechnology is using living things to make useful products that can help people and other living things[14], [15]. It can also be described as, 'Using biology to solve practical problems'. The combined use of biochemistry, microbiology, and engineering to use microorganisms, tissues, and cells for technology is called biotechnology.

History of Biotechnology

Biotechnology has been around for a very long time, since the beginning of human history. Biotechnology has grown in two phases: Classical Biotechnology and Advanced Biotechnology. Classical Biotechnology is the traditional method developed by our ancestors using bacterial fermentation, like making ghee from milk. In the same way, making curd, wine, beer, vinegar, etc., all involve using biotechnology to ferment the ingredients. In Ayurvedic medicine in India, 'Asava' and 'Arista' are made using different substances and the flowers of the Mahua or Dhataki plants. This method has been used since ancient times. In each of these ways, different things are changed into different new things. Advanced biotechnology is different from traditional biotechnology in two main ways. The first is that it allows scientists to use technology to change genetic material and create new products for specific needs. This is done through a process called Recombinant DNA Technology. Ownership of technology and how it affects society and politics. Right now, regular industries like farming and medicine are focusing on making products using biotechnology. New or modern biotechnology uses genetic modification, cell fusion, and fermentation technology to apply to a wider range of industries.

Biotechnology as Interdisciplinary Science

Biology and biotechnology are different because they work on different levels. Biologists usually work with very small amounts of material, but biotechnologists making vaccines might need a bit more. Some projects need a lot of material, like kilograms or tons. Biotechnologists' main goal is to make biological processes bigger and cultivate a lot of microbes and cells. They also work on other parts of the process, like before and after cultivating. These processes have been divided into five main parts choosing and improving strains, growing a lot of cells, making cells work their best, doing the process, and getting the products out. Many areas of biotechnology come from biology and engineering, biochemistry, biophysics, cell biology, colloid chemistry, embryology, ecology, genetics, immunology, molecular biology, medical chemistry, pharmacology, polymer chemistry, thermochemistry, and virology working together. Modern biotechnology has created many tools using knowledge from biology. Figure 1shows how biotechnology involves different areas of study.

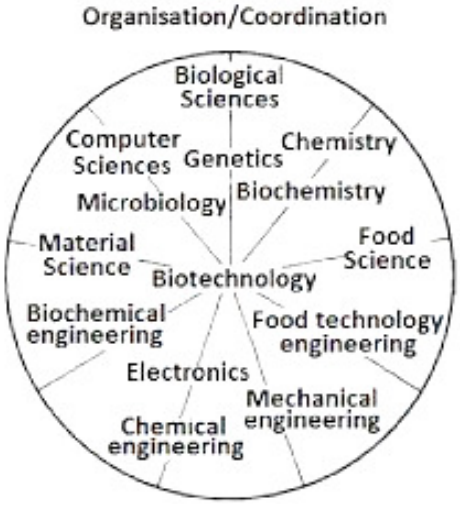

Figure 1: Representing the interdisciplinary nature of biotechnology [Research Gate.Net].

Biotechnology in India

Following the worldwide use of rDNA technology, the Indian government created a new agency called the National Biotechnology Board (NBTB) in 1986. This agency operates under the Department of Science and Technology (DST). NBTB was changed to a whole department called the Department of Biotechnology (DBT). It is now under the Ministry of Science and Technology and is responsible for planning, promoting, and coordinating different programs. Today, India has many agencies like DBT, DST, CSIR, ICAR, ICMR, ICGEB, and IARI that work for the government. These companies and other industries make Biotech products and sell them after testing them on people. The Government has created a Technology Development Board (TDB) to help promote and test new products. TIFAC made a plan called 'Vision 2020' that includes biotechnology. Since the 1980s, India has been helping the biotechnology industry and its products a lot. Right now, there are more than 30 companies in India making modern biotech products. It shows a basic drawing of biotechnology, genetic engineering, and how they are used in different ways. Humans have changed the genes of different kinds of animals and plants for a long time by picking which ones to breed together, instead of letting them naturally mate. In recent times, mutation breeding has used chemicals or radiation to make a lot of random changes in plants or animals, in order to choose the ones with desirable traits.

Genetic engineering is when people change DNA outside of where it is normally found. In 1953, Watson and Crick discovered that DNA is shaped like a twisted ladder. Genetic engineering is when scientists combine different genetic materials by putting them into viruses, bacteria, or other vectors, and then putting them into an organism where they don't naturally occur. This allows the new genetic material to keep reproducing in the organism. Until the early 1970s, biochemists had a hard time studying DNA, which is a molecule in cells. Very long and same-looking chemically, the string of building blocks that make up an organism's genetic material could only be studied indirectly, by looking at the proteins or RNA it produces or by analyzing its genes. But now, technology has made it easy to study the DNA of a cell's macromolecules. Now we can pick out a specific part of a gene and make a lot of copies of it. We can also figure out the order of its building blocks. In the most advanced time for creating new things, machines are making DNA sequences very quickly, non-stop. Using certain methods, a single gene can be changed and put back into the reproductive cells of an animal or plant. This makes it a useful and inheritable part of the organism's genetic makeup. In 1978, someone from the United States. The company 'Genetech' used a method called genetic engineering to make human insulin using a bacteria called Escherichia coli. In 1996, scientists in Scotland successfully created the first clone lamb named 'Dolly'.

Afterward, many animals were copied in 2001, and the sequence of the Human Genome was finished by March 2003. Scientists from the Clonaidcompany in France said they created a clone baby named 'Eve' on December 27, 2002. In May 2005, scientists in South Korea made stem cells using a method called therapeutic cloning. In this method, scientists cloned human embryos, like they did with Dolly the sheep, and then they got stem cells from them. The removed stem cells could be grown in a lab and used later. Bioinformatics is a mixture of biology and computer science. It helps scientists store, share, and analyze biological data for medical research and other fields of science. Bioinformatics uses lots of data from experiments that look at genes and how they are used in living things. Database projects collect and organize information and then share it on the internet. Studying this information helps scientists find new things and ways to help people with medical treatments. In medicine, bioinformatics is used for many important things. For instance, it is used to find connections between genes and diseases, to guess how proteins are shaped from amino acids, to help create new drugs, and to customize treatments for patients based on their DNA.

Applications of Biotechnology

Modern biotechnology uses scientific methods like genetic engineering to improve the natural abilities of living organisms to produce things. Bacteria, like Escherichia coli, are making hormones that are found in mammals, like insulin and somatostatin. Yeast cells were changed to make a vaccine for Hepatitis B. Myeloma cells and B-cells from immunized mice were combined to make hybrid cells that could divide and make antibodies. Hybrid cells called hybridomas are now being used to make monoclonal antibodies. Biotechnology is very important in some areas. Our mission is to help those in need and make the world a better place for everyone. Health care has used biotechnology to get the most benefits. Biotechnology makes new kinds of drugs using proteins and polypeptides. Examples include Humulin (used for diabetes) and Recombivax HB (used for hepatitis B). Biotechnology is creating new ways to improve food and farming. Food biotechnology helps to solve food problems and can also help to prevent diseases like Diabetes and Arthritis by providing better nutritional options.

One of the most important events in human history is when scientists produced the Human Genome Sequence. The Human Genome Project (HGP) is a global research project.

Scientists in labs all over the world have almost finished sequencing the human genome and creating a map of the chromosomes. They are working together to do this. The study of human chromosomes was finished on March 2, 2003. Scientists found around 33,000 functional genes in humans. Over 97% of genes don't do anything. They don't make any protein chains. The goals of the human genome project are to create a detailed map of the genes in the human body, figure out the exact sequence of our DNA, store all this information in a database, find all the genes in our body, and deal with any ethical, legal, and social issues that come up. They also want to do similar studies on other living things. Pollutants in the environment can now be broken down more easily with the help of biotechnology. Bioremediation methods have been proven to be successful in fighting pollution.

Bioremediation means using tiny living things to clean up pollution in soil and water. The bad stuff in the air can make people sick. Certain tiny organisms like bacteria and fungi can break down oil, pesticides, and fertilizers. These organisms belong to groups like Pseudomonas, Micrococcus, Bacillus, Candida, Cladosporium, Torulopsis, and Trichoderma. Genomics is the study and design of genes using computers. Genomics is about figuring out the complete set of genes in a living thing. Proteomics is studying the proteins in the genome using a computer. Proteomics is the study of all the proteins in an organism's genetic code. Using Proteomics and Genomics, we can find new molecules that can work together with other substances. This helps us understand how living things work. Bioinformatics is a new area of biotechnology that involves using information technology. Bioinformatics uses math, statistics, and computer science to help us learn more about biology, biochemistry, and biological data. Bioinformatics has been really successful in sequencing the human genome using a method called shotgun sequencing.

CONCLUSION

Animal cell culture technology is very important in the field of life sciences. It helps us study how cells grow and change, and how we can change their genetics. It's an essential tool for researchers. Doing it right needs special technical skills. This chapter explains the important methods of growing animal cells and how they are used. Animal cell culture technology is really important in the study of life sciences today. It helps us understand how cells grow and change, and also allows us to change their genetic makeup. You need to have special technical abilities to do it well. This chapter explains the important methods of growing animal cells in a lab and how they are used. This chapter talks about the basics of growing animal cells in a lab and how people are using this technique in new ways. The main goal is to help students learn the basics of cell culture and how to work with cell lines. This chapter explains different kinds of cell growing, the liquid used for growing cells, using blood in cell growing, checking if cells are alive, and how cell growing is important for medicine.

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CHAPTER 2

UNDERSTANDING THE BASIC PRINCIPLES OF GENETIC ENGINEERING

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ABSTRACT:

Genetic engineering is when scientists change DNA on purpose to make changes to living things. In the late 1900s, scientists figured out ways to combine pieces of DNA using chemicals and bacteria or viruses. They can also inject DNA directly into cells. In the last few years, new methods have been added to the old ones to create new living things, called synthetic biology. Genetic engineering brings up a lot of important ethical questions. In farming, some experts are worried about the possible risks to people's health from genetically modified plants and animals. They also have concerns about how animals are treated and the impact on the environment from genetic engineering. In medicine, people have argued about whether it's okay to use treatments to make someone's body work better or just to make it work differently. Also, experts who study moral principles have been looking at the possible health risks for humans that come from changing genes in embryos, which is different from changing genes in the body. Finally, when it comes to making babies, some people say that genetic engineering brings up ethical concerns. This includes checking and changing embryos to get rid of or add certain medical or beauty traits.

KEYWORDS:

Bacteria, Genetic Material, Genetic Modification, Genetic Engineering, Restriction Modification.

INTRODUCTION

Microscopic organisms and other Prokaryotes have controlled the Limitation Adjustment (RM) framework found in life forms and help as an instrument of defense against remote DNA, borne by Bacteriophages. These living beings have Confinement Proteins, moreover called Confinement Endonucleases or REs, which cut dsDNA at particular focuses into parts, which are at that point corrupted assist by other endonucleases. Approximately one-fourth of known Microbes procure RM frameworks and of those around one-half have more than one sort of framework. The groupings recognized by the confinement chemicals are exceptionally brief. The nearness of Methyl bunches makes a difference in arranging to avoid the devastation of its claimed DNA by the limited proteins. The endonucleases cleave internal/non-terminal phosphodiester bonds. Limitation endonucleases cleave inner phosphodiester bonds as it were after recognizing particular groupings in DNA which are as a rule 4-6 base sets long, and regularly palindromic. Nuclease could be a sort of protein that can cleave nucleic acids. The nucleases are generally positive in activity; hence ribonucleases act as they were upon Ribonucleic Acids (RNA) and deoxyribonucleases upon Desoxyribonucleic Acids (DNA). A few proteins have a common activity, for illustration, Phosphoesterases can be called Nucleases and are found in both Vegetation and fauna. There are two major sorts of nucleases based on their location of activity, specifically the

Exonucleases and Endonucleases [1]. Exonucleases are competent for evacuating nucleotides one at a time from a DNA particle though endonucleases work by cleaving the phosphodiester bonds inside the DNA atom.

The confinement proteins are nucleases that part as it were those DNA particles in which they recognize specific subunits. There are four sorts of limitation proteins are recognized on the premise of their structure, location specificity, and cofactors. Hence a few RE part the target DNA atom at arbitrary destinations, but others part the atom as it were at the acknowledgment location at a settled separate from the acknowledgment location. Chemicals cleave as they were methylated DNA with frail arrangement specificity. Limitation Chemicals (RE) play an essential part in recombinant DNA (rDNA) innovation or Hereditary Building (GE). The acknowledgment groupings in DNA vary for each confinement chemical, creating contrasts in the estimate, grouping, and introduction of a sticky conclusion (5′/3′end) 'Overhang' of protein confinement. There are diverse chemicals utilized in DNA control, one of them is nuclease, and others are DNA polymerases, kinases, antacid phosphatases, and topoisomerases. The nuclease has significant parts in different DNA repair frameworks, which include DNA replication, base extraction repair, nucleotide extraction repair, bungle repair, and twofold strand break repair [2], [3]. The basic and mechanical distortions of these nucleases may lead to hereditary insecurity or extreme immunodeficiency. Hence, auxiliary science and 3D basic data of nucleases are giving imperative bits of knowledge into atomic designs, DNA acknowledgment, and cleaving instruments.

Genetic engineering requires multiple copies of the DNA sequence or gene under study for any modifications to be made. In earlier times, the process of creating multiple copies of something involved harnessing the power of bacteria and their DNA. The bacteria will replicate and produce multiple copies of the desired item. First, the vector is separated and its DNA is opened using a specific enzyme. Then DNA from the organism being studied is added and it is also opened using the same enzyme. The bacteria are then infected with the changed vector. If we can choose the right bacteria group with the DNA we want, we can find a lot of vector molecules with the same sequence. These molecules can be released by using enzymes. Pieces of DNA are found by sorting them based on their electrical charge and weight using gels. The DNA of the vectors and bacteria is usually between one to ten thousand pieces, and there aren't too many of them so we can find them easily by using a simple staining technique [4], [5]. This technique usually involves a substance that sticks to the DNA and glows when we shine ultraviolet light on it. More fragments from complex organisms make a blurry mark with certain dyes.

One way to identify a particular sequence on the gel is to label a known fragment with an isotope or dye, using DNA's ability to pair adenine with cytosine and guanine with cytosine. The marked molecules are called probes. This is also used to find the differences in genes in living things, either for simple research or to find mutations that cause diseases. Breaking down DNA using different enzymes and putting the pieces back together helps make a map of the DNA's structure. Tiny pieces may be copied, and then the chemical makeup of the pieces' sequence is checked. After that, the pieces are put together to make the final gene sequence. Once the order of the DNA is known, scientists can use a method called PCR to make a lot of that DNA in a test tube. In this method, a small amount of DNA is copied many times using a special heat-resistant enzyme. The process involves heating and cooling the DNA multiple times [6], [7]. This can even be done with just a single cell's worth of DNA. This method is also used in finding out what is wrong with someone's health.

In 1941, scientists found out that DNA is the main genetic material, and about 10 years later, they figured out its structure. This marked the start of DNA science and technology. But, it

was not until the 1970s that scientists started changing DNA using special enzymes, like restriction endonucleases and DNA ligases. In 1972, scientists at Stanford University and the Bay Area did experiments with DNA. They used genetic engineering to put together pieces of DNA from different bacteria to create a new DNA molecule. This was one of the first times this had been done. The new DNA was put into a type of bacteria called Escherichia coli so that it could make copies and carry out the genes that were already there. This well-known example is the first time DNA technology was used to create a modified organism. In simple terms, genetic engineering is the way scientists change the genes of an organism to make it produce something it wouldn't normally make or to make it work better. First, the DNA segment or gene that is wanted is taken from an organism by removing and cleaning the entire cellular DNA. The DNA is changed in a lab and put into a carrier molecule so it can be given to the host strain. The way genes are delivered depends on the kind of living thing and can be grouped into viral and nonviral ways. Changing genes in bacteria or lower eukaryotes can be done using methods like transformation, transfection, transduction, and conjugation. These methods help deliver genes and transfer DNA. Being able to distinguish between the cells that have undergone genetic changes and those that have not is an essential aspect of genetic engineering. This stage typically involves identifying observable distinctions between cells with genetic mutations and cells without genetic mutations. Sometimes, it can be really hard to find a few altered cells in a big group of normal cells. When this happens, it takes a lot of effort to find the altered cells.

DISCUSSION

Invention of Host Controlled Restriction Modification (RM)

The RM system was first found by S. Luria and M. were researchers Human (1952-1953) This refers to a person in the years 1952 and 1953. They found out that a virus called Bacteriophage can change when it grows inside a bacterium and then infects another bacterium. The RM system is found in bacteria and other simple organisms and helps protect against foreign DNA carried by viruses called bacteriophages. Later, J Weigle and G worked together. Bertani and other scientists found out that restriction happens when the modified virus DNA is broken down by specific enzymes in the recipient bacteria. More HO Smith discovered HinDII, the first known Restriction Enzyme (RE), that can be used to create restriction maps. The hard work and studying of these enzymes in a lab helped scientists learn how to control and change DNA, which laid the groundwork for genetic engineering. In 1978, W. became a new member. Arber, D would be rewritten to Arber, the letter D. Nathans and H. O are two companies. Smith won the Nobel Prize in Physiology or Medicine for their important work on restriction modification. 3 shows how the RM system controls Horizontal Gene Transfer [8], [9].

The way the host controls and changes bacteriophages are easiest to see when they move from one type of bacteria to another. Phage was tested on two different types of E. coli bacteria, called strain C and strain K. The phages cannot infect the Escherichia coli K strain. When phages from the infection of E. coli K are put back on E. coli K, they are not stopped. But if they go through E. coli C first, they are stopped again when put on E. So, when a virus spreads to a certain type of bacteria, it depends on the type of bacteria it was last spread on. This change in a strain that can't be passed down to offspring and doesn't have any limits is called modification. The limited phages attach to specific hosts and inject their DNA as usual. When the phage is marked with 32P, it is easy to see that its DNA breaks down shortly after being injected. The enzyme responsible for this breakdown is called a restriction enzyme. The host needs to protect its DNA from the harmful effects of the restriction enzyme, so it has to change its DNA in the right way. The researchers studied how well E. coli bacteria can

control and change the Phage virus using a method called Efficiency of Plating (E. OP) The change happens when certain parts of the DNA are methylated and this affects the way the restriction endonuclease recognizes the DNA. Even though we used phage infections as an example, these processes can happen whenever bacteria transfer DNA to each other [9]. Conjugation, transduction, transformation, and transfection are also limited by the host's control. The genes that control how hosts limit and change their DNA may be part of the host's chromosomes or they may be found on a Plasmid or Prophase 1 (P1).

Types of Restriction Modification

There are four different types of restriction modification systems Type I, Type II, Type III, and Type IV. They all have RE and Methylase Activity, except for Type IV which does not have Methylase Activity. They were named in the order they were found, but the Type II system is the most commonly used in biotechnology. Type I is the most complicated restriction-modification system. They consist of 3 parts: R (Restriction), M (Modification), and S (Specificity). The new complex can break and modify DNA and needs ATP to do it. The breaking usually happens far away from where it recognizes the DNA. The Type II restriction-modification systems are the easiest and most common systems. The Methyltransferase and Endonuclease are not combined into one complex. Instead, they are encoded as two separate proteins and work on their own because there is no specific protein to join them together. The Methyltransferase is one piece, adding methyl groups to one strand at a time. The Endonuclease is a protein that works in pairs and helps to cut both strands of DNA at a specific location near the recognition sequence. So, Type II systems are used in labs to study DNA and make copies of genes. Type III restriction-modification systems have proteins called R (Res) and M (Mod) that work together to modify and cleave DNA [10], [11]. Methylation happens only on one side of DNA, unlike most other known mechanisms. The combination of R and M Proteins competes with itself by changing and limiting the same reaction. Type IV systems are not real RM systems because they only have a restriction enzyme. Unlike other Type IV restriction enzymes, these enzymes only cut DNA that has been changed.

Functions of Restriction and Modification System

The Neisseria meningitidis has many Type II restriction enzymes that are used in a natural genetic transformation process. This process allows a bacterial cell to take up DNA from another bacterial cell and combine it with its DNA. Restriction modification is a major reason why Meningococci can't breed with other bacteria and evolve into new species. The restriction-modification systems can work for themselves and force the cell to keep them by killing any cell that tries to get rid of them. Some viruses have found ways to get around the restriction modification system by changing their DNA with methyl or glycosyl groups, which stops the restriction enzymes from working. Some other viruses, like Bacteriophages T3 and T7, have proteins that stop restriction enzymes from working. Many simple organisms have created various kinds of restriction-modification systems.

Applications of Restriction Modification System

The mutant sequence has REases that work in the same way as the wild-type sequence. Gene therapy: Scientists are looking at a system in bacteria to make vaccines and treatments for viruses in humans. This is because the system helps bacteria fight off viruses. Studies on REases and Zinc Finger Nucleases (ZFN) have found that they can cut the DNA of different human viruses (HSV-2 and HIV-1). The goal is to cause changes and abnormalities in the viruses that infect humans. ZFNs are great for changing the DNA in a living thing because they can find and cut specific parts of the DNA. Zinc finger nucleases are like very precise

scissors for the DNA in our cells. Restriction modification systems can be copied and checked in plasmids because the methylation enzyme helps them resist. Restriction enzymes are used to check the makeup of DNA to see if any changes might impact how well the enzyme can cut the DNA. When we look at normal genes and changed genes using different enzymes, the pieces of DNA we see on the gel will be different lengths. This is because the changed genes won't be cut like the normal genes. RM systems play a big role in the way Mobile Genetic Elements (MGEs) and their hosts evolve together [12], [13]. Genes in plasmids, prophages, transposons, Integrative Conjugative Elements (ICEs), and integrons are mobile genetic elements that can move between prokaryotic genomes using natural transformation, vesicles, nanotubes, and gene transfer agents.

Genetic Engineering in Animal Systems

Genetic engineering means changing an organism's genes using technology. It is a group of methods used to alter the genes of cells, including moving genes between different species, to create better or new organisms. New DNA is made by taking the genetic material we want and either copying it or making it from scratch using special methods. A construct is made and used to put this DNA into the host organism. Paul Berg combined DNA from the monkey virus SV40 and the lambda virus to make the first recombinant DNA molecule in 1972. The process can also remove genes, not just add them. The new DNA can be put into the genome either in a random way or at a specific place. An organism created using genetic engineering is called Genetically Modified (GM) and it becomes a Genetically Modified Organism (GMO). Herbert Boyer and Stanley Cohen made the first GMO in 1973. It was a type of bacteria. Rudolf Jaenisch made the first genetically modified (GM) animal by putting new DNA into a mouse in 1974. The first company to work on changing genes, Genentech, started in 1976 and began making human proteins. Human insulin made in a lab was created in 1978, and insulin-making bacteria were sold in 1982. Modified food that has been changed in a lab has been sold since 1994 when the FlavrSavr tomato was released. The FlavrSavr was made to last longer without going bad, but most other genetically modified crops are changed to be able to resist bugs and weed-killing chemicals. GloFish, the first genetically modified organism made to be a pet, was sold in the United States in December 2003. In 2016, salmon that were changed with a hormone to help them grow bigger were sold. Genetic engineering has been used in many areas, like research, medicine, making products, and farming [14].

In research, GMOs are used to understand how genes work through different experiments like turning genes off, turning genes on, tracking genes, and seeing how genes are used. We can make animals get the same diseases as people by taking out certain genes. Genetic engineering can make hormones, vaccines, and other drugs. It also has the potential to cure genetic diseases with gene therapy. The methods used to make drugs can also be used to make things for laundry detergent and cheese. Genetic engineering is a key tool for scientists, and creating transgenic organisms is a big part of studying how genes work. "Genetic information from many different creatures can be put into bacteria to change and store it. This makes the bacteria genetically modified. " Bacteria are inexpensive, easy to grow, make copies of themselves quickly, can be changed easily, and can be kept at very cold temperatures for a very long time. Once a gene is found, it can be kept in the bacteria to use for research whenever it's needed. Living things are changed at the genetic level to help scientists understand what certain genes do. This could change how the organism looks or behaves, depending on where the gene is used or how it works with other genes. These tests usually include losing a function, gaining a function, keeping track of something, and showing how something works. Genetically modified organisms or GMOs are animals or tiny living things that have been changed using genetic engineering. When genes from a different

type of animal or plant are put into another type, the new organism is called transgenic. If an organism is made using genetic material from the same species or a species that can naturally mate with it, it is called cisgenic. If scientists use genetic engineering to take genes out of an organism, the new organism is called a knockout organism.

Cells are made up of different substances like carbohydrates, fats, DNA, and proteins. But when it comes to genetic engineering, DNA is the main thing that gets changed. The central dogma of molecular biology says that DNA helps make more copies of itself and tells genes how to work. This is important for how all living things work. Genes make messenger RNAs, which then make proteins. So, by changing DNA, we can alter the way proteins and enzymes are made and work. This idea is used in many genetic engineering methods like making new proteins and changing proteins. In addition, enzymes are responsible for controlling and carrying out almost every activity in cells, like the chemical reactions and pathways that make up a living organism's metabolism. So, by changing the way a cell's metabolism works, we can create new metabolic activities and capabilities. This is called metabolic engineering. We often change the DNA to do metabolic engineering. The first genetically modified product allowed by the US Food and Drug Administration for commercial production came out in 1982. It was a type of E. Coli Researchers changed coli to make human insulin. Before this, insulin mostly came from animals or dead human bodies [15], [16]. Insulin is made up of two small parts connected by two special bonds. Unfortunately, regular E. Coli cannot do a lot of protein changes after it's made, like making the disulfide connections needed to make insulin work. To fix this problem, they used bacteria to make early synthetic insulin. They linked the polypeptide chains together using a chemical reaction. But now, they use yeast instead of bacteria to make insulin because yeast can make insulin that is almost exactly like human insulin without needing any chemical changes. Genetic engineering led to the production of other biopharmaceuticals including human growth hormone and tissue plasminogen activator after the successful creation of human insulin. They were produced in the same way as insulin.

Due to the large number of uses and big possibilities of genetic engineering, it is important to consider bioethics. Worries about using genetic engineering in a bad and dangerous way came up when gene cloning and DNA technology started in the 1970s. This happened mostly because people didn't know much about the new technology. Scientists can change the genes of living things, and this has made a lot of people worried about genetic engineering. Although many people think that genetic engineering has a lot of good uses in farming, medicine, and industry, there are still a lot of worries about the ethical and moral issues it raises. These concerns have been around since genetic engineering started and are still talked about today. Because of this, all genetically modified products made anywhere in the world have to be checked and approved by the government before they can be sold. No matter what you're using genetic engineering for, you have to be very careful and responsible when working with genetically modified organisms. We need to make sure we handle, treat, and get safely rid of these organisms. This article is about how genetic engineering is used in biotechnology. It explains the basic and practical ideas about the methods and techniques used in genetic engineering. Our main focus will be on altering the genes of bacteria, specifically the widely recognized bacteria E. Coli is a good choice for study because it has well-known genetics, grows quickly, and is easy to work with.

CONCLUSION

The science of biotechnology utilizes living organisms to manufacture goods or modify processes. It can also be just called biotech. The use of living organisms or their components to develop new products is the essence of biotechnology. Biotechnology includes brewing

and baking bread as they both involve the use of yeast to produce the end products. There are two primary distinctions between advanced biotechnology and traditional biotechnology. Recombinant DNA Technology can modify genetic material to produce novel products. 2It has a big impact on society and politics because of the technology it uses. Genetically engineered organisms are commonly referred to as genetically modified organisms or GMOs. The term transgenic is used to describe an organism that has had genetic material from a different species implanted into it.

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CHAPTER 3

CLONING VECTORS: PLASMID, COSMID, AND PHAGEMIDS

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ABSTRACT:

A cloning vector is a small DNA fragment that originates from a virus, bacteria, or a cell of a more complex organism. It can accommodate additional foreign DNA for the cloning process. The vector contains components that allow for the simple insertion or removal of a DNA fragment. Plasmids are small, circular pieces of DNA that can make copies of themselves using the host cell's DNA replication process. Plasmid vectors are simple. They have a starting point for making copies of themselves inside the host. A bacteriophage, also called a phage, is a virus that infects and multiplies inside bacteria and Archaea. Bacteriophages are made of proteins that protect their genetic material and come in many different shapes and sizes. Their genetic code may contain only 4 genes or up to hundreds of genes. Phagemids are like plasmids, but they have a special part called Origin of Replication that lets them copy single-strand DNA from a virus called bacteriophage F1. Escherichia coli keeps a plasmid as a molecule with two strands of DNA because of a gene called plasmid ORI.

KEYWORDS:

Artificial Chromosomes, Base Pair, Cloning Vector, Foreign DNA, Shuttle Vector.

INTRODUCTION

The cloning vector is a minute DNA segment that is extracted from a virus, bacteria, or a cell of a more complex organism. It can hold onto a foreign DNA piece for cloning. The vector contains specific components that facilitate the insertion and removal of DNA fragments. The small DNA segments created can exhibit either blunt or sticky ends. The joining of vector DNA and foreign DNA can occur through molecular ligation when their ends match. A plasmid is a self-replicating, small DNA molecule that exists separately from the cell's main DNA. Plasmids are typically tiny, circular DNA molecules in bacteria, although they can also be present in archaea and eukaryotic organisms at times. In nature, plasmids usually have genes that help the organism survive and give advantages like being resistant to antibiotics. A cosmid is a special type of plasmid that has a part of a Lambda phage in it. They are often used as a tool for making copies of genes in genetic engineering. Cosmids can be used to make collections of genes from an organism. Collins and Hohn first talked about them in 1978 [1], [2].

Cosmids have between 37 and 52 kilobytes of DNA, which is based on the size of the normal bacteriophage packaging. They can copy themselves as plasmids if they have the right place to start copying: for example, SV40 ori in mammal cells, ColE1 ori for making double-stranded DNA copies, or f1ori for making single-stranded DNA copies in simple cells. They often have a gene that makes them resistant to antibiotics, so they can be easily found and tested by growing them on a substance with antibiotics. The cells that did not accept the

cosmid would not be able to grow. A phagemid or phasmid is a type of DNA tool used for copying genes. It has features of both a bacteriophage and a plasmid. These vectors have a beginning point for copying DNA from both a plasmid and a bacteriophage. Phagemid vectors are different from regular plasmids because they can be put into a bacteriophage's capsid. This is because they have a genetic code that tells the bacteriophage to package them [3], [4]. Phagemids are used in many biotechnology applications. One possible application is in the field of molecular biology, where they could be utilized in a method known as "Phage Display." In this lesson, you will learn about different types of vectors like plasmid, cosmid, phagemids, YAC, BAC, shuttle vectors, yeast vectors, and minichromosomes.

The discipline of genetic engineering depends substantially on the use of cloning vectors, which are critical tools for manipulating DNA. A cloning vector is a short section of DNA produced from a virus, bacterium, or a higher organism's cell that acts as a stable carrier for foreign DNA fragments during the cloning process. The vector has unique properties that allow for the easy insertion and removal of DNA segments. DNA fragments produced during cloning may include blunt ends or overhangs, referred to as sticky ends. Molecular ligation, which joins compatible ends of vector and foreign DNA, is then used. The resultant constructions, known as recombinant DNA, may be further modified. Following initial cloning, a DNA fragment may be subcloned into a more specialized vector designed for particular use. Genetically modified plasmids are one of the most often used cloning vectors. However, different vectors serve different functions, and their selection is determined on the properties of the DNA being altered. Escherichia coli is the most common host organism for cloning, and vector types include plasmids, bacteriophages, cosmids, and bacterial artificial chromosomes [5], [6].

For bigger DNA segments that cannot be successfully maintained in Escherichia coli, yeast is used as an alternate host. Yeast artificial chromosomes (YACs) are cloning vectors that can accommodate DNA pieces up to 300,000 nucleotides long. Artificial chromosomes, known as YACs, BACs, or human artificial chromosomes (HACs), have the potential to transport far bigger DNA pieces than existing vectors. YACs and BACs, which may contain DNA pieces as long as 300,000 nucleotides, need three structural components: a replication origin, a centromere, and telomeric end sequences. Recent advances in genetic engineering have resulted in significant improvement in the manipulation of both bacterial and eukaryotic DNA. DNA isolation, enzymatic cutting at specific sites, and reconnecting DNA from two species are now possible. Cloning and expression vectors are key components of recombinant DNA technology. Recombinant DNA is created by cloning foreign DNA, which may be extracted from the genome, chemically manufactured, or obtained as complementary DNA (cDNA) utilizing mRNA [7], [8].

The use of vectors is essential for cloning because a distinct DNA molecule is needed to link the foreign DNA. Vectors, which transport foreign DNA segments, may take the form of plasmids, bacteriophages, cosmids, phagemids, transposons, viruses, YACs, or BACs. In molecular biology, vectors serve a critical function in transporting foreign genetic material into host cells for replication and expression. plasmids, viral vectors, cosmids, and artificial chromosomes are examples of common vectors. The most often used vectors are plasmids, which include a replication origin, a multicloning site, and a selectable marker. Expression vectors, which are intended to express transgenes in the target cell, include a promoter sequence. Transcription vectors, which can be transcribed but not translated, are utilized to amplify the insertion. Shuttle vectors are intended to be maintained in both bacterial and non-bacterial hosts, such as yeast or mammalian cells. The insertion of vectors into target cells is known as transformation in bacteria and transfection in eukaryotic cells. Expression vectors,

transcription vectors, and cloning vectors differ in their specialized tasks within the greater domain of genetic engineering, with each performing a separate role in the modification and expression of genetic material.

DISCUSSION

Characteristics of Cloning Vectors

Cloning vectors often include features required for their propagation and maintenance in Escherichia coli, such as a functioning Origin of Replication (ORI). Many plasmids have the ColE1 replication origin. All cloning vectors have characteristics that make it easy to insert or delete a gene from the vector. This might be a Multiple Cloning Site (MCS) or polylinker, which has several distinct restriction sites. The restriction sites in the MCS are first cleaved by restriction enzymes, and then a PCR-amplified target gene digested with the same enzymes is inserted into the vectors using DNA ligase. The target DNA sequence may be put into the vector in a specified direction if desired. If required, the restriction sites may be employed to subclone another vector. 2. Other cloning vectors may employ Topoisomerase instead of ligase, which allows for faster cloning without the requirement for a restriction digest of the vector or insert. In this 'Topo Cloning' approach, a linearized vector is activated by attaching Topoisomerase I to its ends. This 'TOPO-Activated' vector may then receive a PCR product by ligating both of the PCR product's 5' ends, releasing the Topoisomerase and producing a circular vector in the process [9], [10].

DNA recombination is another technique of cloning that does not utilize DNA digest or ligase, such as the Gateway Cloning System. The gene, once cloned into the Cloning Vector (called Entry Clone in this procedure), may be easily introduced into a variety of expression vectors by recombination.

The vector carries a selectable marker that allows the selection of favorably transformed cells. Antibiotic resistance is often employed as a marker, with the Beta-Lactamase Gene, for example, conferring resistance to the Penicillin group of beta-lactam antibiotics such as Ampicillin.

Some vectors have two selectable markers, such as the Plasmid pACYC177, which contains both the Ampicillin and Kanamycin resistance genes. Shuttle vectors intended to be maintained in two distinct species may also need two selectable markers, while certain selectable markers, such as resistance to Zeocin and Hygromycin B, work in various cell types. Auxotrophic selection markers that enable an auxotrophic organism to grow in minimum growth media may also be utilized; examples are LEU2 and URA3, which are used with their respective Auxotrophic Yeast Strains.

Reporter genes are utilized in certain cloning vectors to aid in the screening of successful clones by using properties of these genes that make successful clones clearly identifiable. Such characteristics in cloning vectors may include the IacZa segment for complementation in blue-white selection, as well as marker or reporter genes in frame with and surrounding the MCS to enable Fusion Protein synthesis. GFP and Luciferase are two examples of fusion partners that may be employed for screening. A cloning vector does not have to have components necessary for the expression of a cloned target gene, such as a promoter and Ribosomal Binding Site (RBS); nonetheless, many do and may then function as an expression vector. The target DNA may be inserted into a position controlled by a specific promoter required for the production of the target gene in the selected host. Where the promoter is present, the gene's expression is ideally tightly regulated and inducible, ensuring that proteins are only created when needed. Some vectors, such as those used for in-vitro mRNA

synthesis, are exclusively intended for transcription and do not produce any heterologous proteins [11], [12]. These vectors are known as transcription vectors. They may lack the sequences required for polyadenylation and termination, making them unsuitable for protein synthesis.

Types of Cloning Vector

There are many different cloning tools to choose from, and the best one depends on factors like the size of the thing you want to clone, how many copies you need, and how you plan to clone it. Big pieces of DNA may not stay in place very well in a regular cloning tool, especially if there are a lot of copies of the DNA. So, we might need a more special tool to clone big pieces of DNA. Examples of cloning vectors include Plasmids, Bacteriophages, P1 Vectors, Phagemids, Cosmids, Bacterial Artificial Chromosomes (BAC), Yeast Artificial Chromosomes (YAC), Human Artificial Chromosomes (HAC), and Retroviral Vectors.

Plasmids

Plasmids are small circular pieces of DNA found outside the main genetic material in a cell. They can make copies of themselves using the cell's own equipment. Plasmid vectors have a part that lets them replicate on their own inside the host. Plasmids are in a lot of bacteria, like Escherichia coli, and a few other organisms, like Yeast. Bacterial plasmids can be passed from one bacterium to another, or they can't be passed. Conjugative plasmids help bacteria share DNA with each other and can quickly spread to many bacterial cells in a group. Examples of these plasmids include F Plasmid, many R, and some Col Plasmids. Non-conjugative plasmids, like R and Col plasmids, don't transfer DNA through conjugation. Bacteria have DNA from their genes and small DNA molecules called plasmids. Plasmids with special features are often used in the lab for copying DNA.

These plasmids usually can't transfer genes to other cells, but they have many other abilities. One important feature is a place where different enzymes can cut and insert a new gene. Bacteria with plasmids can make extra DNA that can copy itself on its own. These are the basic cloning tools that are used often. Many plasmids can be used to copy DNA pieces that are up to 15 kilobases in size. One of the first cloning tools used a lot is the pBR322 plasmid. The pUC series of Plasmids is one example of alternative cloning tools, and there are various options available for Cloning Plasmid Vectors. Plasmids like pUC19 have many copies in each cell, around 500-700 copies. Having many copies is good because it makes more of the recombinant plasmid for future changes. Low copy number plasmids are better to use in some cases [13]. For example, when the protein from the gene being cloned is harmful to the cells. The pUC plasmids feature a region for inserting various genes, as well as a gene that confers resistance to the antibiotic ampicillin in bacteria.

Characteristics of Plasmids

Plasmids were the original instruments utilized for transporting and replicating genes. They are DNA molecules that occur naturally and can make copies of themselves without any outside help. Not all plasmids are round from the beginning. They can be found in bacteria, archaea, and eukaryotes. Plasmids are usually between 1,000 and 250,000 base pairs long. DNA pieces up to 10 kilobytes can be copied into plasmids. The plasmids have a lot of copies, which helps make more of the modified plasmid for future experiments. The low number of plasmids are used when a gene makes a toxic protein in the cells. The sole purpose of plasmids is to contain the genetic code necessary for replicating their proteins. These genes that make protein are close to the start of DNA replication. Some examples are pBR322, pUC18, F Plasmid, Col Plasmid.

Bacteriophage

A bacteriophage, or phage, is a virus that infects and multiplies inside bacteria and archaea. Made up of proteins, bacteriophages can encase either DNA or RNA and can have either simple or complex shapes. Their genetic makeup could consist of as few as 4 genes or as many as hundreds of genes. Viruses called phages make copies of themselves inside a bacterium after their genetic material enters the bacterium's cytoplasm. Bacteriophages are prevalent and diverse in the world. Bacteriophages are viruses that can be found everywhere that bacteria are. Over 1031 bacteriophages are believed to exist on the planet, surpassing the total number of other living entities like bacteria. Viruses called phages are found in places where there are a lot of bacteria, like soil and the intestines of animals. Seawater contains a lot of viruses, such as phages. In microbial mats at the surface, there can be up to 9 x 10^8 virions per milliliter of water. And up to 70% of the bacteria in the ocean may be infected by phages. They have been used for more than 90 years instead of antibiotics in the past in the US, Central Europe, and France. They are thought to be a potential treatment for bacteria that don't respond to many different drugs [14], [15]. The bacteriophages utilized for cloning go by the name's phage lambda and M13 phage. Due to the limited capacity of DNA that phages can hold, unnecessary genes may have to be removed to introduce new DNA into the phage. For instance, the genes responsible for lysogeny may be removed as we are exclusively utilizing the lytic cycle for phage cloning.

There are two types of phage vectors; Insertion Vector and Replacement Vector. Insertion vectors have a special place where foreign DNA, which is 5-11 kilobytes in size, can be put in. In replacement vectors, the cleavage sites surround a region with genes that are not necessary for the lytic cycle. This region can be removed and replaced with the desired DNA during cloning. The inserted DNA can be larger, ranging from 8 to 24 kb. Phages can only carry DNA that is a certain size. If the DNA is too small, the phage cannot carry it properly. This property can be used for choosing things. If the vector is too small without adding anything, it may not be selected for spreading. Phage Lambda is a kind of virus that has a genome of about 48502 base pairs in size. A third of the bacteriophage genome isn't required, making it possible to remove and substitute it with new DNA during cloning. It can only combine 4-5 thousand base pairs of donor DNA. The Phage has a head, a tail, and small fibers on its tail. The genetic material is made up of 48. There are 5,000 base pairs in the DNA, while the single-stranded DNA has 12 base pairs and sticky ends on both ends. Because these ends work well together, they are called Cos Sites [16], [17]. Getting sick from a virus called phage needs the virus to stick to the outside of a cell, then the virus's tail to get smaller, and lastly the virus to inject its DNA into the cell.

P1 Phage Vector

This cloning system uses the Bacteriophage P1 as a base. The P1 cloning system offers functionality that lies between that of Cosmids and YACs.Fragments of DNA put into P1 vectors are usually between 75 and 95 kilobases in size, but they can be as big as 100 kilobases. Cloning for P1 can produce around 105 duplicates for every small piece of vector and inserted DNA.The P1 cloning vector has two P1 DNA origins of replication. One ensures that there is only one copy of the plasmid DNA for each host chromosome, while the other has the capability to produce 20 copies per cell once activated. In addition to the selectable markers, the vector also contains the pac site and two loxP sites from phage P1.Recombinant molecules are put into packages using different P1 packaging extracts in a test tube. Packaging starts when a cut is made at the packaging site. DNA is put into a P1 phage head in a specific way, where 100-115 kilobytes of DNA can fit inside. The initial cut for packaging occurs at the packaging location, and the subsequent cut is made at a predetermined distance

from the packaging site, but there is no specific order for it.The straight DNA is put into the receiving bacterium. DNA molecules must have two loxP sites to form a circle and undergo replication within the bacterium.The loxP sites are like little marks on the DNA that a protein called Cre can attach to and make changes.

Phagemids or Phasmid Vectors

Sometimes it is necessary to create a single strand of DNA for tasks like sequencing DNA and making specific changes to the DNA at certain sites. A group of cloning tools were made using a type of DNA called bacteriophage M13, which has only one strand. The bacteriophage Fl is similar to M13, both viruses infect Escherichia coli. Phagemids are special plasmids that have a place for a virus to attach and make copies of its DNA. Escherichia coli keeps a plasmid as double-stranded DNA because of a gene called plasmid ORI. When Escherichia coli cells get a virus called F1 phage, it turns on the F1 virus. It switches to a replication process and produces individual DNA strands. These strands are then packaged into virus particles and released from the infected cell. A special kind of DNA called phagemidpBlueScript II KS (+–) is used to make single-stranded DNA. Phagemids, which are produced in a laboratory, contain a component from the F1 phage that aids in their replication.They are frequently utilized alongside the M13 phage to facilitate cloning. They can act like a plasmid and are packaged as single-stranded DNA in virus-like particles.

Cosmid Vectors

Cosmids are plasmids containing unique Cos sites. An example of Cosmid is pJB8. A plasmid is modified by introducing lambda DNA with the Cos site in order to produce this Cosmid. Cos sites are a special sequence that is cut by a specific enzyme called Phage endonuclease during the process of rolling circle replication. The vector is 5. 4 kilobytes big For putting DNA into a virus shell in a lab, the DNA has to be between 37 and 52 kilobases in size. If the DNA is too small or too big, it can't fit into the protein coat. This makes empty heads instead of full viruses. Cosmids are like plasmids and can take in the bacteriophage DNA segment. This part of the DNA has special sites called Cos sites that help pack the DNA into the λ phage. They are also put into packages. This allows new DNA or genes from another organism to be put into the host organism using transduction. The physical map shows a Cosmid named pJB8 that is 5. 4 kilobases in size. Cosmids are special plasmids that have a piece of bacteriophage DNA with a special site called Cos, which helps in packing the DNA into particles. It is usually used to make copies of big pieces of DNA that are between 28 and 45 kilobases in size. A Cosmid is a kind of mix of a plasmid and a lambda phage Cos sequence. The DNA sequences come from the lambda phage. Cosmids can be used to create collections of a cell's genetic information. Collins and Hohn described them in 1978 for the first time. Cosmids can hold 37 to 52 kilobytes of DNA, but usually they hold 45 kilobytes. This is because of the size of the normal bacteriophage packaging.

They can make copies of themselves if they have the right starting point, such as SN40 for mammal cells, colE1 for double DNA copying, or F1 for single DNA copying in bacteria. They often have a gene that helps to pick out the transformed cells, like antibiotic resistance. This makes it easier to find the transformed cells by growing them on a substance containing the antibiotic. The cells that didn't absorb the cosmid wouldn't be able to grow. Unlike plasmids, they can also be put into phage capsids, which help move the foreign genes between cells through transduction. Plasmids can become unsteady when too much DNA is added to them because their bigger size makes them more likely to mix and change with other DNA. To go around this, we use phage transduction instead. This is possible because of the cohesive ends, also called Cos sites. This means they are like using the lambda phage as a

carrier, but all the lambda genes are removed except for the Cos sequence. The Cos sequences are about 200 building blocks long and very important for packaging. They have a place where DNA is cut by a protein called terminase, every 12 bases. This makes the circular cosmid have two sticky ends that are 12 base pairs long. The DNA has to be in a straight line to fit inside a phage head. The CosB site holds the terminase and helps it cut and separate the strands. After the previous cosmid is packaged, the terminase holds the CosQ site of the next cosmid to stop it from being broken down by cellular DNases.

Bacterial Artificial Chromosomes (BACs)

Bacterial Artificial Chromosomes (BACs) resemble Escherichia coli plasmid vectors. They include ORI and genes that encode ORI-binding proteins. These proteins are necessary for BAC replication. It is generated from the naturally occurring F plasmid. The DNA insert size ranges between 150 and 350 kb. This suggests that inserts of up to 350 kb may be cloned in Bacterial Artificial Chromosomes (BAC). BACs are maintained in Escherichia coli at a copy number of one per cell. BACs are based on F plasmids, whereas PACs are based on P1 phages. One example of a BAC is 'pUvBBAC'. It is an artificially manufactured plasmid measuring 11827 bp in size. It is a variant of the bacterial F plasmid with a cloning limit of 35–300 kb. The marker genes are the chloramphenicol-resistant gene and the lactose-metabolizing gene, lacZ.

Yeast Artificial Chromosomes (YACs)

Yeast Artificial Chromosomes (YACs) are capable of copying and transporting large segments of DNA, up to 200 kilobases in size. They are used to make copies inside certain types of cells. These work as chromosomes in a cell with a nucleus. It has yeast telomeres on both ends. A special sequence called Yeast Centromere is there to make sure the cells divide properly during Meiosis. The ORI comes from bacteria. Both yeast and bacterial cells can be used for hosting.

Human Artificial Chromosome (HAC)

The Human Artificial Chromosome (HAC) may be beneficial in inserting genes into human cells and examining the operations of human chromosomes. It can hold a lot of DNA (there's no limit on size), so it can clone big pieces without trouble. It also doesn't cause mutations when it integrates into the host's chromosomes like viral vectors do. HAC is a man-made chromosome used to carry human genes. A small amount of DNA is used to build HAC so that chromosomes can work properly. It allows genes to add specific sequences. There is no restriction on cloning and it can replicate big pieces of DNA. They are used a lot to study how genes are expressed and to find out what the human chromosomes do.

Retroviral Vectors

Viral vectors are viruses that have been changed to carry genetic material in a non-harmful way. They still have the ability to make the genetic material into proteins using their own machinery. However, because viral vectors often do not have everything, they need to infect cells, they need helper viruses or packaging lines to help them infect a lot of cells at once. Viral vectors are made to put the insert into the host's genes permanently. The insertion of the new gene results in distinct genetic indicators within the host's DNA. For instance, retroviruses leave a unique pattern when they insert into a host's genome, and this can be detected as a sign that the viral vector has become part of the host's genetic material. Retroviruses are viruses that have RNA as their genetic material. Retroviral vectors are used to put new or changed genes into animal or human cells. The virus's RNA is changed into

DNA using reverse transcriptase and then becomes part of the host cell. You can put any gene you want into the retrovirus. This special gene can be added to the cell's chromosomes and stay there. They are frequently employed to study and learn about genes that cause cancer and other genetic traits in individuals.

Expression Vector

Expression vectors make proteins by first copying the genetic material in the vector and then turning it into protein. They need more parts than other vectors that only copy genetic material. Using different living things to make something would need different parts, even though they all need similar things, like a starter for making copies, a place for the cells to read the copies, and signals for when to stop. For example, the Eukaryotic Expression Vector pSG5 is 4100 base pairs long. Essentially, we modify a Eukaryotic vector to operate within a Prokaryotic cell. This new version is called Expression Vector and helps with reading genes.

Shuttle Vector

Often a plasmid, a shuttle vector can thrive in two different host environments. So, when DNA is put into a shuttle vector, it can be tested or changed in two kinds of cells. The best thing about these vectors is that they can be changed in Escherichia coli, and then used in a system that is harder or slower to use, like Yeast. The shuttle vectors are plasmids that can work in both simple cells and complex cells, like yeast and bacteria. For example, they can work in E. coli and also in other bacteria like Rhodococcuserythropolis. There are also special viruses that can move between bacteria and mammals. Shuttle vectors are often used to make many copies of a gene in Escherichia coli quickly. They can also be used for experiments done in a lab and making changes, such as altering genes or doing a specific type of experiment called PCR. One popular kind of shuttle vector is the yeast shuttle vector. Most of the commonly used Saccharomyces cerevisiae vectors can be used to transfer DNA between different types of cells. Yeast shuttle vectors are made with parts that make it possible to copy and pick in both E. coli cells and Yeast cells. The Escherichia coli part of a yeast shuttle vector has a start-up point and a marker that can be chosen, like antibiotic resistance, beta-lactamase, or beta-galactosidase. The Yeast shuttle vector has three parts: a sequence that makes copies of itself, a center point to help it divide, and a marker that helps the yeast grow. One example of this marker is URA3, which is a gene that helps the yeast make a certain enzyme. Shuttle vectors can stay alive in two types of living things, and have two sets of instructions for making copies of themselves, one for each type of living thing, and two sets of instructions for choosing which ones survive, one for each type of living thing. Researchers want to transfer genes from one organism to another. The shuttle vector can move between different organisms, but it needs to have certain features to survive.

The shuttle vector needs to have a replication starting point for both organisms, because these are sequences that are identified differently by proteins from different species. If we want to use the shuttle vector in a cell like Yeast, it needs to have a centromere sequence. This helps the cell divide properly. Finally, we need to use two different markers, one for each organism. Antibiotics mostly only work on bacteria and don't affect other types of cells like yeast. If you want to use yeast for the shuttle vector, you have to use a different marker to select for it. Many times, we use strains that don't work well in some ways the body processes things. If the yeast doesn't have one enzyme to make a certain amino acid, it can't grow unless it gets that amino acid from outside. If the working DNA for the enzyme is on the shuttle, then the yeast can grow without needing the amino acid. The way cells work is different if they have the vector compared to those that don't have it. Many well-known shuttle vectors have been used to change thermophiles that need to live without oxygen. All of these vectors

have genes that make them resistant to antibiotics. These genes come from certain types of bacteria. Vector pIKM1 is made up of parts from different plasmids. It has a gene called mls from pIM13 and a kanamycin resistance gene from plasmid pKD102 in Streptococcus faecalis. It is based on the pUC19 plasmid. Gene knockout tools were made using pIKM1 for studying genes.

CONCLUSION

A vector is a number that shows both size and direction. Vectors are helpful in many different situations, like when we need to know about force or how fast something is moving. In physics, vectors are often used to find how far something has moved, how fast it's going, and how quickly its speed is changing. The new DNA is copied and used by the cell to make proteins. It makes one DNA copy into a lot of copies. Cosmid and Phagemid contain different types of sequences. Cosmids are a mix of a cos site and a plasmid, which makes them a hybrid vector. Phagemids are special plasmids that have a part from a virus called F1 phage. This part helps them copy themselves. The purpose of cosmid vectors is to duplicate extensive DNA segments and replicate their DNA in a manner reminiscent of a virus or a small, circular plasmid. Cosmid vectors are used to combine two plasmids in a single cell. They can grow in both bacteria and animal cells.

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CHAPTER 4

EXPLORING THE METHODS OF GENE TRANSFER IN ANIMALS

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ABSTRACT:

Molecular biology is now experiencing a rapid influx of discoveries. One thing they can do is create new animals with special traits. An animal that has undergone genetic alteration through the addition of genes from another species or through gene editing techniques for specific traits is referred to as a transgenic animal. By intentionally manipulating genes, we can transform the attributes of animals. The initial successful genetic modification was performed on a mouse. After that, pigs, sheep, cows, and rabbits arrived a couple of years afterward. There are different methods utilized to ready foreign genes for use in animal transgenic techniques. The gene we want is put into different types of containers like yeast chromosomes, bacterial plasmids, and cosmids. Many methods, like heat shock, electroporation, viruses, the gene gun, microinjection, and liposomes, are used to put the vector with the special gene into the host cell. Transgenesis involves introducing new genes into reproductive organs, eggs, and embryos through methods such as DNA injection, viral delivery, stem cell manipulation, and cell replication. Currently, the best marker for transgenic organisms is fluorescent protein. Even though people have worries about transgenesis, this review focuses on the basics of putting genes into animals and how it's used in business, healthcare, and farming.

KEYWORDS:

Animal Cells, ES Cells, Gene Transfer, Gene Therapy, Genetic Material.

INTRODUCTION

In the 1970s, scientists were able to put new DNA into plant and animal cells in a lab. In the early 1980s, scientists were able to change genes in mice for the first time. Scientists have created new types of animals and plants by adding genes from different species. These include amphibians, cattle, chickens, fish, insects, nematodes, pigs, rabbits, and sea urchins. Ways to move genes have been used to try to help people with gene therapy since 1990. Gene therapy is currently mainly focused on treating diseases caused by a single gene or on treating cancer. Until now, only a little progress has been made in the clinic. However, gene therapy is still in the early stages and has a lot of potential for the future. As mentioned earlier, gene transfer methods can be used to create transgenic animals. These animals can be used for two main things for scientific research; and for making new medicines or organs that can be transplanted into humans. Gene transfer in higher organisms can be used for healing people in two main ways by changing the genes of some cells in the body somatic gene therapy, and by changing the genes in reproductive cells [1], [2]. Using gene therapy to change the genes that are passed on to children has not been tried with humans except for adding mitochondria during fertilization.

This is because there are a lot of ethical concerns and there is not much scientific information about it. To make changes to the genes that can be inherited, genetic modification must happen when the organism is in the beginning stages of growth. On the other hand, changes to somatic cells, such as in somatic gene therapy, can involve many different types of cells in the body. Different kinds of cells are talked about in the paper at the right times. This part talks about different cell types that can be used for adding new genes to the next generation of animals. Changing the genes of a newly fertilized egg should make an organism with the same change in most or all of its cells. So, scientists have been mostly studying the zygote for genetic engineering. In this paper, the word "egg" is used to refer to all stages of development from the egg cell to the early embryo before it hatches. Before fertilization, eggs could potentially be used for adding new genes. So far, there have been practical problems that stopped them from being used. Eggs taken after a female's ovulation would need to be fertilized after being genetically changed [3], [4]. This would mean using in vitro fertilization. Eggs that might have been genetically modified would need to go through a long process outside of a living organism. Because this could only be harmful to the eggs, it is hard to see any reason for changing genes at this level instead of at the zygote.

In theory, it might be possible to try changing a lot of pre-egg cells in the body using genetic manipulation. The new special organism will make changed eggs every time it lays eggs, that are ready to be fertilized. However, the necessary technology is not yet advanced enough to support this in vivo method, and no transgenic animals have been created using this approach. Eggs that have been cut are not very good for adding genes. When only one cell is changed, the organism will probably have a mixture of normal and altered genes. This organism would have two types of cells: some with the transgene and some without it. Sure, the more times the cells have been divided before transgenesis, the less likely it is that a high proportion of the cells will be changed in the transgenic. It's really hard to change multiple cells in an egg. More importantly, each changed cell would not have the exact same change, which we will look at later. So, even though the organism would be made up of changed cells, it would still be a mix of different cells. Mosaicism doesn't always cause problems. Mosaic transgenics might make sperm or eggs that have the transgene inside them.

This can make the next generations fully transgenic. However, not all mosaic transgenics have genetically changed reproductive cells all the time. Some transgenic organisms may not have all of their reproductive cells changed. So using post-zygotic eggs would make transgenesis less efficient than using zygote-stage eggs. One big problem with eggs is that it's very difficult to put new genes in specific places in their DNA, because it's hard to find eggs with the right genetic changes. While it has been possible to change genes in eggs, the success rate is not high enough for it to be useful. However, lots of genetic experiments don't need to target specific genes. Transgenes that have been mixed in randomly have been used in many different ways [5]. Some examples of genetic engineering include controlling gene expression using external chemicals, killing hormone-producing tissues using toxic genes, and making transgenic sheep that produce human blood clotting factor VIII in their milk.

Embryonic stem (ES) cells can be obtained by growing cells from the inner part of the early mouse embryo in a laboratory setting. This was discussed by Torres in 1998. In contrast to other cell lines, ES cells maintain their original DNA structure even after being cultured in the laboratory for an extended period. During this time, they can still develop into any type of cell in the body. Additionally, ES cells can travel into and thrive within the embryo. ES cells possess the special capacity to form hybrids with other embryos when they are introduced into blastocysts or combined with morulae.The non-pregnant female mouse has the new embryos placed into her uterus. Roughly 50% are anticipated to develop and be delivered as

robust mice. About half of the babies should have a mix of genes from the parents. ES cells can account for as much as 80% of a mouse's cells and may also encompass the cells responsible for reproduction. ES cells can be changed in a lab dish before they are used [6], [7]. ES cells have a big advantage because they can be exposed to different substances in a lab, which makes it possible to choose specific changes in their genes. This special ability makes ES cells very helpful for gene targeting tests and uses.

ES cell technology has allowed scientists to use a variety of genetic methods in mice. Different ways of changing genes in mice include getting rid of a gene, fixing or replacing a gene, targeting a gene in specific conditions, and using gene trap systems. Thus far, ES cells have not been widely utilized as they have only been effectively created from mice. It would be surprising if this limit is a basic rule in biology. Additional research is required in order to provide ES cell lines to other animal species. The mice utilized for generating ES cells may have the necessary mutations for ES cell creation.It may take an extended period to produce ES cells from animals other than mice if the mutations are necessary for their formation. However, there has been a lot of progress in understanding how cells develop in mammals. This advancement is expected to facilitate the successful establishment of non-mouse ES cells. The mice technology can be applied to modify genes in other species in a lab once non-mice cells are accessible [7], [8]. In 1996, scientists successfully transferred genetically modified sheep cells from one sheep to another. Eggs without fertilization and with removed center received the transferred nuclei at the metaphase stage.

Nuclei were removed from body cells that were stopped from growing by keeping them in a solution with very little nutrients. This stopped the cells from copying DNA and making proteins. The transfer of nuclei from one type of cell to another resulted in some cases of successful embryo growth. The donor cells were capable of altering and becoming adaptable to generate new embryos. Baby animals were born after putting the reconstructed embryos into mother sheep. The lambs' DNA was determined to have been derived from the donor cells after further genetic testing.Nuclei from sheep embryo cultured cells were used in certain experiments. After these important tests, scientists have been able to successfully make copies of animals like cows, goats, and pigs using cells grown in a lab. The chances of changing genes in embryos using nuclear transfer are very important. Genes can be put into cells before making animals with modified genes. In addition, by choosing specific cells from donors, NT can create animals with targeted genes.

DISCUSSION

The movement of genes between living beings can lead to the development of varied characteristics in organisms. This truth serves as the foundation for enhancing vital farming crops, whether through conventional farming practices or innovative biological methods. In both scenarios, individuals alter a biological process to create new organisms with specific characteristics, such as animals with increased muscle mass or disease-resistant corn. The major disparities between old-school farming practices and gene manipulation in laboratories do not stem from their objectives or approaches. It's more about how fast, accurate, and trustworthy they are, and how much they can do. Traditional breeders aim to produce offspring with desired traits by combining numerous genes when crossing two plants or animals. When a sperm and egg come together, each parent gives half of their genes to their baby. However, the genes from both parents can vary in each individual egg and sperm. Additionally, since the desired traits typically originate from a single parent and are influenced by a small number of genes, multiple crosses must be conducted in order for the appropriate gene combination to manifest in the offspring. However, the offspring usually need to be bred with the original variety again to make sure the new trait is firmly

established. Occasionally, when a new organism is created by combining the traits of its parents, it may end up with undesirable traits from one parent and may not inherit the favorable traits from the other parent. There are numerous challenges and difficulties associated with traditional breeding methods.

Molecular biology techniques aid in addressing these issues by enabling researchers to regulate gene transfer at a fundamental level. Scientists can insert individual genes for specific traits directly into a genome, rather than relying on the random mixing of genes. They can also change how the genes show themselves in the new plant or animal. Molecular gene transfer can be used to create improved plant varieties more efficiently than conventional breeding by targeting particular characteristics. Lab techniques for moving genes between organisms use natural methods of gene transfer. These methods are different from the usual way genes are passed on through sexual reproduction. This includes cells taking up DNA and passing genetic material between cells using things like viruses. Scientists started by looking at how things work in simple things like bacteria and the viruses that attack them. Research has been moving really fast. Now scientists can put genes into many different things like soybeans and sheep. We still have a lot of work to do to make gene transfer and related technologies for embryos and plants better. Scientists like to use certain types of bacteria and flies in their research because they are easy to work with and there is a lot of information about them [9]. Model systems are very important for research to move forward. However, scientists who study tiny things in living things need to use their methods to learn more about plants and animals that are important for farming. Moving in this direction will not completely replace the traditional way of breeding crops with the use of molecular gene transfer. It will increase the options for improving important crops.

Bacteria can share genes with each other in different ways. Scientists discovered in the 1950s and 1960s how to use these processes to study how genes are controlled in bacteria. In the 1970s, they also created new ways to transfer genes artificially in bacteria. It was quite simple for some bacteria to absorb fragments of DNA from their surroundings. The genes on the new DNA pieces can be passed down and used to give the bacteria new traits. Scientists came up with special ways to make the DNA get into the new hosts, stay there, and use the genes. Gene transfer is now regularly done in labs with bacteria *like E. coli* is a type of bacteria. The aim of gene transfer experiments with other organisms is to study how genes are controlled and to make sure that the new traits can be passed down to future generations and are always present [9], [10]. These other organisms are more complex than bacteria. So, the issues and methods used in the experiment are very complex. Special tools and conditions are needed to transfer DNA into other cells.

The growth of knowledge in molecular biology has happened because scientists found ways to work with genes as big molecules. Scientists can find, separate, cut, and join genes and move them from one kind of living thing to another. Enzymes come from bacteria and help scientists work with genes from any living thing. These methods are commonly used in molecular biology. The fifth part, gene transfer, needs to be figured out separately for each living thing. Different kinds of animals, plants, and tiny organisms are able to transfer their genes in different ways. Plants have usually been harder to work with compared to animals or microbes. Technology is getting better very quickly, and it's possible that soon, most living things will be able to have genes added to them.

This report looks at the current state of gene transfer systems for animals, plants, and microbes that are important for farming. It also looks at what might happen soon. Scientists have made these methods by studying how molecules work in living things. These processes are used, just like how they were first used for bacteria, to create ways to transfer genes

between different organisms. This allows researchers to study how genes work and give an organism new trait that they want. Several things need to be thought about when creating gene transfer systems. The gene needs to have a noticeable "tag" that can be easily seen so we can track its movement into a new host. Sometimes the different gene is enough. We can find the gene by the new thing it gives or we can see it using a special test for its DNA. Regrettably, it can be challenging to pinpoint things directly due to the time and inconvenience involved. In these scenarios, it is possible to label the foreign gene by linking it to a readily locatable gene in the host. Genetic qualities that provide bacteria with resistance to drugs are frequently employed as markers [11], [12]. Cells that have these genes can survive when given medication, while cells without the genes will die.

Another thing to think about is how well genes can be passed from one organism to another. The gene's likelihood of being identified must be high for the transfer to be successful. If medicine becomes less effective, or if other methods of choosing are used, a lower amount may be okay. In these situations, lots of cells can be treated to put new genes in them. However, only a few of these cells actually keep the new genes after treatment and are saved. Special vectors can make gene transfer work better. Foreign genes stuck to the vector will be taken by it into the host cell. Vectors are usually made from circular DNA molecules called plasmids or from viruses. Different transfer systems are characterized by specific features that could limit the quantity of foreign DNA they are capable of transferring. Segment sizes are determined in base pairs, which are the fundamental building blocks of DNA. A regular gene can have between 1,000 to 50,000 base pairs. Some methods can move big sections of DNA, but most can only move small ones. Scientists are trying to create better tools that can carry more than one gene at once because they want to transfer multiple genes at the same time.

Sometimes when a DNA molecule is used to carry other genes, its size can limit how much extra DNA it can hold. Short vectors, which are less than 10,000 base pairs, are more limited than long vectors, which can be over 100,000 base pairs. However, bigger vectors need additional changes to add foreign DNA to them. It is important to consider the location of foreign genes within the host cell at the conclusion of the process. Genes can be kept on independent, self-replicating "minichromosomes," or they can be added to the larger chromosomes of the host cell to maintain them. Depending on why the experiment is being done, it might be better to either keep the new genes separate or add them into the existing genes. However, to make sure that the genes passed on to the next generation of animals or plants are reliable, they need to be joined with the existing genes. The number of identical copies of a foreign gene that end up in a cell is called gene copy number, and it is related to the state of genes. Depending on what the experiment is for, you may want a lot of copies or just one. For instance, if we use gene transfer to change a cell line so it can make a lot of a valuable protein, we would use a minichromosome that can copy itself many times. However, if the goal is to give an animal or plant a new gene to help it fight off diseases, it may only need one or two copies of the gene in its own chromosomes. This will make sure the gene is expressed correctly and passed on to future generations.

Genes that are moved must be controlled to make sure the proteins they produce are made in the right amounts, at the right time, and in the right location. Genes are usually regulated by specific sequences in the nearby DNA. These ordered sets are influenced by different things inside cells, like hormones. Genes that are moved can be controlled by their usual sequences that regulate them. Instead, scientists can give them new instructions to copy nature or make different things happen. Before making permanent changes to an organism's genes, it's important to study the gene and understand how it normally works. This research is easily

done using a "transient expression" system. This system helps to quickly measure the activity of transferred genes in cells, without having to wait for long-term changes in the cells' genetics. The speed and adaptability of early studies are improved by "shuttle vectors," which can replicate in both animals or plants and in bacteria. Scientists can use shuttle vectors to grow and isolate genes from bacteria in large amounts. They can then change the genes in a test tube and move them into animal or plant cells to see how they work. The relocated genes can also be extracted from the animal or plant cells, inserted into bacteria, and then cultivated in large quantities for additional applications.

Placing DNA directly into the liquid in which animal cells are cultured is the oldest and most widespread technique for introducing genetic material in a lab.DNA and bacteria both require entry into a cell and retention in order for the cell and its descendants to utilize the new genetic information for developing a new trait. The structural differences between animal cells and bacterial cells lead to variations in their functionalities.Bacterial cells are distinguished by having both a membrane and a wall, while animal cells only have a membrane. In order for the DNA to penetrate the cell, the organisms' robust cell wall must be eliminated. The nucleus houses the majority of the genetic information in animal, plant, fungal, and yeast cells and has its own membrane.Living things with cell nuclei are called eukaryotes. New genetic material usually needs to go through a second membrane to be added to a eukaryotic cell permanently. Simple bacteria called procaryotes don't have a nucleus and can easily take in new DNA. The main benefits of direct DNA uptake are that it's easy to do and can be used for many different organisms and cells. Many cells can be treated at the same time, instead of putting DNA into one cell at a time, which takes a lot of effort and time [13], [14]. Direct uptake is very helpful for studying gene expression in cell culture because it is quick and easy. These studies help to understand what a gene does before scientists try to change the gene in living animals or plants, which takes a lot of time and effort.

Genes from another organism are put into a new organism and start working in the new cells after a short time, usually 1 or 2 days. The DNA is taken up by the cell to quickly show what new genes do. This is called "transient expression. " For longer studies, the genes need to become part of the cell's own chromosomes or be carried in by new chromosomes to make sure they are passed down reliably. The foreign genes are added into the animal cells in large numbers, so integration happens a lot. Moreover, transferring genes directly into cultured cells to make genetically modified proteins is used to make products for sale. Cells can make drugs, hormones, and other useful stuff by adding in the right genes. Insulin for diabetics is made in bacteria now. Animals can't always take up things directly because their bodies may not be able to use them properly. Therefore, transferring genes into an animal embryo usually needs to be done differently. Plants can grow into new plants from just one cell, so they are not limited in the same way as other organisms.

Chemical treatments can make animal cells in a lab take in DNA from their surroundings. This happens more often in cells grown in a lab than in animals. Most easily and commonly, cells are mixed with DNA that has been mixed with calcium phosphate and then made into small particles. This treatment makes the DNA smaller, so cells can accept many copies of the foreign genes. Or, DEAE-dextran can be used to help DNA enter cells. Cells grown in a lab are not very specialized and don't control their genes as well as normal organs in an animal do. Scientists have made a way to put DNA into organs like the liver or spleen of animals without hurting them. We put calcium phosphate-precipitated DNA directly into the organs with a small number of enzymes to help the DNA get inside. This method helps scientists to study how a single gene works in specific cells of an organ in an animal, which

gives a more accurate understanding of the gene's function. Another way to change organs is to inject a certain kind of DNA into an animal's belly, where it is absorbed and used by the liver and spleen tissues.

It has been hard to change plant cells using chemicals, but people have recently made some important progress in doing so. Polyethylene glycol has been used to help protoplasts from different plants take in and keep DNA stable. The plants included wheat, grass, oilseed rape, tobacco, and petunias. DNA uptake directly into plants happens less often than when DNA is carried by a vector, but it can happen in any type of plant cell. However, protoplasts are used as the receivers, so they need to be able to grow into plants in order to create genetically modified species for farming. Insects, fungi, yeast, and bacteria cells can all be treated with different chemicals to help them take in DNA directly. Typically, direct uptake is used to bring in DNA molecules that contain modified genes. Putting foreign genes inside the cell is called direct uptake. Vectors can help these genes become part of the cell's chromosomes. Protoplast fusion, also referred to as somatic fusion, is a technique for manipulating the genetic makeup of plants. The process combines two distinct plants to create a new plant with characteristics from both. The name of this newly created plant is somatic hybrid. Hybrids are made by mixing different types of the same kind of plants or by mixing two different kinds of plants. Somatic fusion can be used to make potato plants strong against potato leaf roll disease [10], [15]. The sick potato plant that doesn't grow many potatoes gets combined with a wild potato plant that can't grow potatoes. The wild plant is able to resist the sickness. The new plant has a mix of chromosomes from both parent plants, making it similar to polyploid plants.

A retrovirus is a kind of virus that puts its own genetic material into the genetic material of the cell it infects. This changes the cell's genetic material. When the virus gets inside a cell, it makes its RNA into DNA using a special enzyme. This is different from the usual way cells make DNA. The new DNA is added to the host cell's genome by a special enzyme called integrase. This makes the retroviral DNA become a provirus. The host cell thinks the viral DNA is its own and uses it to make the proteins needed to make more viruses. To make it more likely that a gene will be used, it is transferred using a carrier like a virus or plasmid. Retroviruses are often used to carry genes into cells because they can easily infect the cells. The babies born from this method are a mixture of cells, not all of them have the retrovirus. The transgene can only be passed on if the retrovirus becomes part of the reproductive cells.New types of tools have been created for gene therapy. These tools can permanently change the genes in cells by putting new genetic material into the cells' chromosomes. Long-term applications of this technology may include its use in genetic disease treatment and research efforts. For example, it can correct genetic defects in stem and progenitor cells. Our retroviral vectors have been designed to selectively target different cellular types. More than 300 clinical trials have employed gamma retroviral and lentiviral vectors in the search for treatments for a range of diseases. Retroviral mutations can be used to create mice that have cancer so that scientists can study how cancer spreads.

CONCLUSION

The process of gene delivery involves introducing genetic material, such as DNA or RNA, into cells.The genes will only function if the genetic material, which can be DNA or RNA, penetrates the host cells. Despite not being completed, the success of gene delivery depends on the stability of the new genetic material within the host cell. Additionally, the foreign substance has the potential to integrate into the genetic material or replicate independently. In genetic engineering, a gene gun is a tool used to put new DNA, RNA, or protein inside cells. Sonoporation is using sound, usually really high-pitched sound, to change how easily

substances can pass through a cell's outer layer. This method is often used in molecular biology and non-viral gene therapy to help large molecules like DNA or RNA get into a cell. This process is called transformation or transfection. Sound waves are employed in sonoporation to aid in the transportation of large DNA or RNA molecules into cells. There are multiple approaches for delivering the gene into the cells. The use of molecular tools and technologies simplifies and enhances the process of introducing genes into cells without causing damage or altering their function. Furthermore, the advancements in molecular biology have made it easier to transfer genes more accurately into specific cells. Gene technologies have helped to find cures for many diseases. Employing gene transfer in disease treatment leads to improved long-term outcomes.We need to exercise patience and give it time for the invention of new, cost-effective, and more secure gene transfer techniques. This will make treating diseases easier and cheaper for everyone.

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CHAPTER 5

ANIMAL CELL CULTURE MEDIA: UNDERSTANDING THE IMPORTANCE OF MEDIA FOR CELL GROWTH

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ABSTRACT:

This section discusses the significance of culture media in providing a controlled environment for the growth of microorganisms, cells, and small plants. It explains the distinction between the fluid utilized in animal and plant cells and the one utilized in bacteria and fungi. This is about how important cell culture is in life sciences. It involves growing cells, tissues, or organs in labs. The chapter talks about how culture media used in microbiology allows all types of bacteria to grow, instead of just specific ones. One important thing we talked about is choosing the right stuff for cells to grow in, like what it's made of and what it needs to grow. This includes things like sugar, protein building blocks, vitamins, minerals, blood serum, hormones, and things that help the cells stick to the container. This lesson explains how culture media helps control the cell cycle and gives cells the best environment to stay alive. It talks about things like pH, osmolality, and how to make and clean culture media. Various methods for cultivating cells in the laboratory are explored, such as hanging drop, suspension, and monolayer.In addition to investigating new cell lines, we also examine established ones. This unit helps people understand how to make a fake environment that helps cells grow. It provides instruction on the fundamental principles of cell culture media in the life sciences industry.

KEYWORDS:

Animal Cells, Amino Acids, Cell Culture, Cell lines, Culture Media.

INTRODUCTION

Essential nutrients such as sugar, amino acids, salts, and vitamins are present in cell culture media.with each having a specific purpose. It can be purchased from stores in either powder form or liquid form. We will discuss the functions of each component in the cell culture liquid in the following section. Buffering systems play a role in regulating the pH to create optimal conditions for cellular growth and division. The two kinds of buffering systems employed in cell culture media include the natural buffering system and the CO2 buffering system.Cells with a natural buffering system need to be kept in an environment with 5-10% CO2, usually in a CO2 incubator. HEPES is good at keeping the pH level steady between 7.2 and 7.4, and it doesn't require a specific gas environment. However, this chemical buffer can be very harmful to some types of cells at higher amounts. Cultural media that you can buy usually have phenol red in them. This helps you keep an eye on the pH of the media. The cell culture liquid is a bright red color at pH 7.4, which is the best pH level for growing cells. When the pH is low, the phenol red makes the liquid yellow. When the pH is high, it makes the liquid purple [1]. However, there are some drawbacks to using phenol red to measure pH. It has the potential to interfere with flow cytometric studies and disrupt the equilibrium of sodium and potassium levels in the body. In the cell culture media, inorganic salt is essential

for regulating water balance and sustaining cellular energy through the provision of crucial ions such as sodium, potassium, and calcium [2].

Amino acids are the basic parts of proteins and are needed for cells to grow in a lab. Moreover, it is important to add essential amino acids to the cell culture liquid because cells cannot make them by themselves. For example, L-glutamine is an important amino acid that gives nitrogen to NAD, NADPH, and nucleotides. It also gives extra energy for doing metabolic activities. Additionally, the liquid that cells grow in can be improved by adding amino acids that are not essential for growth. This helps the cells grow better and live longer. Carbohydrates in the cell culture food give energy to the cells. Many cell cultures contain sugars like glucose, galactose, maltose, or fructose in their media. Proteins and peptides like albumin, transferrin, and fibronectin are often used in cell culture media. Albumin can bind to and remove harmful substances from the cell culture media because of its ability to do so. Just like that, Fibronectin helps cells stick together, while Transferrin carries iron to the cell membrane. Fats and oils are important for growing cells in serum-free media [3]. We add vitamins to the media to help the cells grow. Tiny amounts of zinc, selenium, and copper are called trace elements. Usually, serum-free media have added things to help cells grow well and keep enzymes working right. Antibiotics are often used to stop the growth of bacteria, fungus, and mycoplasma in cell culture. Using too many antibiotics can be bad because it can mess up how your cells work. The cell culture media that companies sell have added important nutrients like amino acids, carbohydrates, proteins, fats, vitamins, minerals, hormones, growth factors, and small amounts of certain elements. Special liquid made from the blood of baby and young cows is often used to help cells grow in lab dishes [4].

Cells require serum to obtain the necessary nutrients for their proliferation. Serum has important substances that help cells grow and work properly. It can also add proteins like albumin and transferrin to the media, which help transport other molecules into the cell. Albumin transports fats, vitamins, and hormones in the body. put inside the tiny parts of the body. It also adds proteins like fibronectin to the culture media to help cells attach to the surface. It also adds substances to the cell food that helps the cells to spread out before they start to multiply. It also adds protease inhibitors to the culture media to protect cells from being broken down by proteases. It also adds small amounts of minerals like sodium, potassium, zinc, and iron to the culture media. It makes the liquid thicker and helps protect cells from being harmed when the liquid is stirred. It also helps control the acidity of the culture media, so there's no need to add any other acid control [5]. Therefore, as shown above, serum in a medium has everything cells need to grow and reproduce. The serum might have things that stop growth. Adding serum makes it more likely to get contaminated. Adding serum can make it harder to purify and isolate cell culture products. Companies that sell cell culture medium offer it in three different forms: powdered, liquid, and ready-to-use. The powdered form needs to be mixed and cleaned before it can be used. This is the cheapest cell culture liquid, but it needs to be filtered before using it to make sure it's clean. After the media has been filtered and made free of germs, you can add horse serum or fetal bovine serum to it [6].

This type of cell food needs to be mixed with water before using. This culture media can be used as it is without any extra steps needed. No matter what kind of liquid is used to grow cells, it needs to be tested to make sure it doesn't have any fungus, protozoa, or bacteria in it. This is done by putting it in a warm, CO2-filled container for three days before using it. Store cell culture media in a cool, dark place. Sterilization means getting rid of all living things like germs and other tiny organisms from things like food and medical equipment. Sterilization means making something completely clean by using heat, chemicals, radiation, pressure, or a

filter. Sterilization is different from disinfection, sanitization, and pasteurization because those methods only reduce the number of germs and other living things, while sterilization gets rid of all of them. After sterilization, an object is called sterile or clean. This is the most common way to make things germ-free in labs. Autoclaving uses steam under pressure to sterilize things. This method kills germs by using high heat and water to break down their proteins. This strong heat comes from the steam under pressure, which has a higher amount of heat compared to water. This heat is released when it touches a cooler surface, allowing it to quickly heat and penetrate dense materials for sterilization. Cooking with dry heat, like using flames or baking, is not the same as cooking with wet heat, like using an autoclave [7]. Dry heat methods don't use water, so proteins can't be broken down. Instead, heat without water can destroy or kill harmful germs by breaking down their cells.

This needs more energy than breaking down proteins, so it's important to use high temperatures when sterilizing with dry heat. For example, you can sterilize something in 15 minutes by heating it in an autoclave at 121°C. But if you use dry heat, you would need to heat it at 160°C for the same amount of time to sterilize it. However, using wet or dry heat to sterilize culture media with delicate parts is not a good idea. Filtration is a way to quickly clean culture media solutions without using heat. The filter sterilization technique uses a filter with very small holes. This lets the liquid pass through but stops any germs from getting through. Membrane filters made from cellulose esters are the most commonly used filters for research. Filters with small holes that are 0.2 mm wide are usually used to get rid of bacteria from culture media. However, very tiny things like viruses and phages can easily go through these filters so filtration is not a good choice if you're worried about them [7], [8]. Ethanol or isopropanol at 60-90% concentration mixed with water can kill germs, but it doesn't work on spores. Ethanol or isopropanol works by changing proteins with the help of water. UV, X-rays and gamma rays are different kinds of radiation that can harm DNA, so they are great for sterilizing things like medical equipment. Natural media is made up of only biological fluids that occur in nature. Natural materials are very useful and good for many types of experiments with animal cells. However, one problem with natural media is that it is hard to make the same thing again because we don't know exactly what it's made of.

DISCUSSION

Animal cell culture means growing and multiplying animal cells outside of the body, as long as they have the right food and conditions. Growing cells in a lab is called cell culture. It is done in a test tube instead of in a living organism. It is about taking cells from animals, performing surgery to remove tissues or organs, and putting them in a special environment to help them grow and survive. A group of cells that are all the same can come from one single cell, which is called a clone. So all the cells in a clonal population have the same genes. Animal cells grow slowly and usually take 18 to 24 hours to divide. This means that when there are only a few bacteria in the animal cell culture, they can quickly grow and take over the culture, causing contamination. Cells need the right temperature, a good surface to stick to, and the right environment to grow well. This includes keeping the pH and osmolality at the right levels in an incubator. Cell culture is a way to study how cells grow and change in a controlled environment. It is very important for research in life science [9]. This technology is now being used in the fields of genetics, immune system testing, surgery, building organs, and making medicines.

In the 1950s, scientists started using animal cells in labs regularly. George Gey was the first person to create human cells from a woman with cervix cancer. These cells, called HeLa, helped scientists make important medical discoveries. Cell culture is important for making a lot of viral vaccines. Cell culture technologies are used in different areas, like testing new

drugs to see if they work and if they are safe, making vaccines and biopharmaceuticals. With improved technology, different types of culture liquids have been created. The culture medium helps cells stay alive, grow, and do their jobs. Different things that help cells grow, like nerve growth factor, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, platelet-derived growth factor, and transforming growth factor, were found. When these things were added, the cells grew more. In 1976, scientists worked faster to create serum-free media [10]. The timeline for when animals developed their own culture throughout history.

The most important part of growing cells in a lab is choosing the right liquid they need to grow well. The kind of cells and what they need will determine which media to use for culturing them. This includes how they grow, change, and make specific products like medicines. A regular culture liquid has vitamins, amino acids, sugar, salts, serum, and hormones to help cells grow. Also, medium helps keep the right balance of acidity and the concentration of particles. Media can be made from natural or man-made materials and used with some natural nutrients to grow animal cells. Media supplements are a mix of amino acids, salts, glucose, vitamins, and other nutrients. The components needed depend on the type of cells being grown, so there are many different types of media to choose from [11]. Some extra parts like hormones and growth factors are needed to help cells grow and stay healthy. These parts are not already in the media and serum.

Serum is very important for cell culture. Serum has a lot of good stuff like amino acids, proteins, vitamins, carbs, fats, hormones, and growth factors. Serum has proteins that can carry other molecules into the cell. Furthermore, serum provides substances that help cells stick to surfaces before they start dividing. Cow serum from unborn and young cows is often used to help cells grow in a lab. However, there are some drawbacks to using serum in media: Serum doesn't have enough of the right growth factors for cells, so it can't be used alone for culturing. It can also have harmful chemicals and substances that stop cells from growing and multiplying. Serum could have a lot of germs like viruses, fungi and mycoplasma that can make you sick. The liquid in the culture dish can make it hard to separate and purify the cells and drugs. To fix this, other methods are used to isolate the cell products. Even though not needed for cell growth, antibiotics like penicillin and streptomycin are often used in culture medium to stop bacteria and fungus from growing [12], [13].

Growing animal cells in a lab involves creating the right conditions like food, temperature, and hormones so the cells can grow well. So, it is important to keep the temperature, balance of chemicals, acidity, and air, provide a surface for support, and protect the cells from harm. CO2 incubators are used to grow cells from humans and animals at a temperature that is like our body temperature. In addition, the cells mostly come from animals that need a warm environment to grow best. The concentration of the solution has a big impact on how cells grow and work. It keeps the cell membranes strong and healthy. If the pressure outside the cell is different from the pressure inside, the cell will either get bigger or smaller. The concentration of the liquid used is decided by the ingredients in it. Both sugar and salt are the main things that affect the concentration of the liquid, even though amino acids also have an effect. In general, most commercial media are made to have an osmolality of about 300 m Osmol. Osmolality can be checked using a device called an osmometer[14], [15].

Cell culture incubators need CO2 from outside to keep CO2 levels steady inside the incubator. To keep the handling area clean, the CO2 tank should be kept outside the lab and the gas should be piped in. The main jobs of the incubator are to keep the inside clean, keep the temperature steady, make sure there is a little bit of CO2 in the air, and keep the air very humid. The liquid is mixed with sodium bicarbonate and carbonic acid to keep it stable, and

the pH level needs to be carefully controlled. A lab that grows cells needs a good microscope with clear optics and the option to take pictures. We can keep an eye on the shape, size, and other features of cells to see if they are stressed. This helps us understand if cells are not doing well. Changes in the way cells look can show if something is wrong with the culture. You can use a good microscope to see signs of tiny germ contamination. Regularly looking at cultures through a microscope can help us from losing important material by noticing problems early. Moreover, when testing sick cells, the results may not be accurate or consistent. When picking a microscope, choose one with a long or extra-long working distance condenser so you can see flasks and roller bottles. Usually, a 20x magnification lens is enough, but it may not show a clear image for things that are not very flat. A good, low-energy, wide-view lens is very helpful for looking at culture colonies [16], [17].

These cells need to attach to a solid surface to grow and multiply in a flat layer. These cells stick to the container they are growing in using a sticky substance called the extracellular matrix. This sticky substance usually comes from tissues that don't move and are part of the connective tissue network, like fibroblasts and epithelial cells. The bottom of the culture dish has a single layer of cells, so we call these monolayer cultures. Most continuous cell lines grow in a single layer. These cells are only one layer, so they can be put on a glass slide and looked at with a microscope. Suspension cells, also known as non-attached cells, don't stick to the surface of the container and float in the liquid. Hematopoietic stem cells and tumor cells are both examples of suspension cells. These cells come from the blood, spleen, and bone marrow. These cells grow quickly and don't need to be changed as often. When a group of cells is transferred to a new container and grown again, it is called a secondary cell culture. When sub-culturing, some cells are moved to a new container with new food to keep them growing. Growing primary cells again creates new cell lines. During the process, the cells that can grow the most become the main ones, making the population more similar in their genetic and physical traits. When cells are regularly grown again, they might change from the original cell.

After the cells are separated and grown in a lab, they can multiply and stick to the surface to form a complete layer. After a few days, the cells grow close together and form a layer. If they grow too close, it can stop them from growing properly and they might die. Right now, the cells need to be transferred to a new container using an enzyme called trypsin to help the cells come off the old container. The cells are taken out and put into a new bottle after spinning them and adding new liquid. This is often called 'splitting' because the cells in one bottle are divided equally into two bottles for growing. A passage number is the number of times a cell line has been grown again and again. To move cells from one place to another, we first remove them from the surface they are attached to with enzymes or by scraping them off. Then we put them in new liquid. Once the cell line is created, we should figure out its karyotype. This will show which type of cells it is and how many changes there are in the chromosomes. Karyotypes can range from mostly normal to aneuploid. Although it's better to have a normal set of chromosomes, having an abnormal set doesn't mean the cells can't be used for studying in a lab. We can still use them if they are still able to function normally. If it's important to have normal cell lines for making transgenic animals, we need to be careful when handling the cells to prevent any changes in their chromosomes. The karyotype (number and appearance of chromosomes) of cell lines is influenced by the species they come from, how they are grown, how they are divided, and if they are frozen.

Extremely cold temperatures (–180°C to –196°C) are needed to keep cells stored. Liquid nitrogen is used to freeze cells at really cold temperatures because it helps prevent ice crystals from forming at temperatures below –130°C. Extreme cold can kill cells by making ice

crystals, changing the levels of electrolytes, and dehydrating them. It can also change the pH level. To stop things from getting frozen, glycerol or Dimethyl sulfoxide (DMSO) are added. Usually, the liquid used to freeze cells has 90% serum and 10% DMSO. Ideally, use healthy cells that are growing quickly and change the liquid they're in a day before freezing. Additionally, the cells need to be cooled down slowly from room temperature to -80°C so that the water can come out of the cells before they freeze. Thaw frozen cells quickly by putting the vial in a warm water bath at 37°C and shaking it gently. This is to reduce the amount of ice and crystals that can harm cells. After heating and melting, the cells can be moved into a container with the right liquid to keep growing. Measuring how many cells are alive is really important in cell culture. The cell viability test can show if the cells in a culture are alive or not. This is needed to find out how well a pesticide or insecticide works, to check for harm from poisons, and to see if a drug could be helpful. Normally, we need to do a cell viability test regularly to see if the cells are still alive or not. If there are a lot of dead cells, the cell mixture should be replaced. There are two kinds of tests to see if something is viable.

The different ways to increase the amount of cells include using spinner flasks for cells floating in liquid and roller bottles with tiny beads for cells that stick to surfaces. They are made of a glass flask with a paddle inside that moves the liquid when it's on a special machine. Commercial versions have one or more extra arms that can be used for taking samples or pouring liquids out. The cells can't sink to the bottom of the flask, so they only bunch up when there are a lot of them in the flask. Mixing the substance makes it easier for gases to be exchanged. The expression 8. 8 times Cells stick to the surface of microcarrier beads in roller bottles, which makes more space for them to grow. These bottles can be used in special machines with attachments that spin the bottles in a certain way. After the bottle spins around completely, the whole layer of cells is quickly in contact with the liquid. Microscopic beads of different sizes, around 150 micrometers or 90-300 micrometers, made of materials like dextran, plastic, glass, gelatin, or collagen can be used to grow a single layer of cells. Growing cells in a single layer on small beads can give a lot of surface area for the cells to grow on, compared to the amount of liquid in the container. This can be up to 90,000 square centimeters for every liter of liquid, depending on the size and weight of the beads. It's also good because the cells can be treated as a liquid mixture.

Microcarrier uses a swinging or sliding tool to mix things in a liquid without breaking the small beads. When the conditions are just right, cells can grow close together in large amounts before they start causing problems. Erythropoietin (EPO) is a hormone made by the kidney when the body doesn't have enough oxygen. This hormone helps make more red blood cells and heal wounds. EPO makes the bone marrow make more red cells, which helps the blood carry more oxygen. EPO helps with anemia in cancer patients going through chemo, treating AIDS, and chronic kidney failure.

Recombinant human EPO (r-HuEPO) has been made using cells from Chinese hamster ovaries. Using r-HuEPO is better than blood transfusion because it doesn't need donors or special transfusion centers, and it lowers the risk of getting a disease from the transfusion. Haemophilia A is a genetic disorder where the body does not make enough Factor VIII, which is needed for blood to clot. Like EPO, Factor VIII is also a type of protein that has sugar molecules attached to it, and it can be made in CHO cells. Hemophilia B, also called Christmas disease, is a bleeding problem caused by not having enough factor IX. Recombinant Factor IX made in cells is used to help people with hemophilia B. Tissue Plasminogen Activator (tPA) is a type of enzyme that helps break down blood clots in the body. It can be used in some patients who have a heart attack or stroke. tPA is the first medicine made using mammal cells. Production and how tPA works.

CONCLUSION

Animal cell culture is when animal cells are kept alive and able to grow in a laboratory using the right food. Cells need the right temperature, pH, and good growth materials to grow well. There are two types of animal cell media: natural media and artificial or synthetic media. Natural media is made from fluids found in living things, like blood and tissue, and can be used to grow many different types of animal cells. Synthetic media is a mixture of different nutrients, vitamins, salts, gases, and other substances. It can be changed to fit different needs and comes in four types: media with serum, serum-free, chemically defined, and protein-free. Cell cultures can be divided into two types: primary cell cultures and secondary cell cultures. Primary cells can grow in a single layer attached to a surface or floating in a liquid. Secondary cell lines are made from primary cells by growing them again. Based on how long they last, we put cell lines into two categories: finite and continuous. Checking if cells are alive or dead is important in cell culture, and is done using tests that look at whether cells can exclude dyes or by testing their metabolism. Cell culture technology is used in many different fields like genetics, immune system studies, gene therapy, making body parts, and making medicine. Growing animal cells in a lab is important for making new medicines and treatments for sick people. It helps scientists understand diseases like cancer and genetic disorders better. This can improve the lives of patients and help them feel better.

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CHAPTER 6

TRANSFORMED CELLS: UNDERSTANDING THE DYNAMICS OF TRANSFORMED CELLS

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ABSTRACT:

In the field of molecular biology and genetics, transformation refers to the process in which a cell accepts external genetic material and undergoes a change as a result. For transformation to happen, the receiving bacterium needs to be ready, which can happen naturally when it's trying to survive in tough conditions, like when there's not enough food or a lot of other bacteria around. It can also be made to happen on purpose in a lab. "Transformation" can mean putting new genes into cells, like in animals and plants. But for animal cells, it usually means turning into cancer, so we call the process "Transfection". Viral transformation is when cells change and grow differently because of new material they inherit from a virus. This can cause them to replicate more than usual. This process happens when a virus changes a living cell or group of cells in a bad way. The phrase can also mean putting DNA into cells using a virus. Viral change can happen on its own or with the help of medicine. Some viral infections like HPV and T-cell Leukaemia virus can cause cancer. Hepatitis B and C are caused by viruses changing the cells in the body. Viral change can also be used in medical treatments. There are different ways to put foreign DNA into a eukaryotic cell. Some use physical methods like electroporation, squeezing cells, using nanoparticles, or magnetofection. Others use chemicals or biological particles like viruses to carry the DNA into the cell. Gene delivery is a step needed for gene therapy and changing the genes of plants. There are lots of ways to put genes into cells and tissues of different living things. Overall, the methods can be sorted into two groups: non-viral and viral. In this lesson, you will learn about changed cells, ways to keep cells alive, how cells are used to make products and fix tissues, and making more cells in bioreactors.

KEYWORDS:

Cells, Genes, Genetic Material, Host Cells, Viral Transformation.

INTRODUCTION

Transformation is when something changes in its appearance or traits because of changes in its genetic material, like DNA or RNA. This can happen spontaneously or be caused on purpose. It affects how genes are expressed. Cells can change by themselves. Cells can change if they get infected by a special virus. Genes can change through gene transfection. Radiations or cancer-causing agents can change something. Usually, cells grown in a lab can easily have changes in their genes or can become unstable. Changes in the genes of cells growing in a lab can happen because the cells grow and divide quickly, or because there are already some altered cells present that aren't getting removed. When a cell can live forever, it's called immortalization. Usually, cells can only divide about 20-100 times before they die. But, tumor cells can keep dividing forever, which is why they can form a continuous line of cells. Scientists have found that the length of time cells can live in a lab is controlled by about

ten strong aging genes. These genes make proteins that slow down the cell cycle [1], [2]. Cells become immortal because certain genes that control the cell cycle, like p53 and Rb, stop working. To make cells live longer, they are infected with special viruses that contain genes to make them live forever before they get old. After that, the cells stop dividing and go into a stage of emergency that lasts for several months. Eventually, some cells can grow and become immortal. When cells change, they can have altered surfaces which can change how they stick together. This can make them have less ability to stick to each other or to other surfaces. These results in losing the cell's need to stick and spread to grow, called anchorage independence. Cells that don't need to be attached to anything often grow in a messy or random way. The changed cells also have a specific feature called loss of contact inhibition. This usually means that the cells don't grow as much and can't hold as much stuff as regular cells. This can be seen by looking at the shape changes in the cells after they have been transformed. The cells may look disorganized and out of place [3], [4]. Cancer cells make a lot of their own growth hormones, so they don't need as much serum to grow. Cells changing into cancer cells is a complicated process. The cells taken from cancerous tumors are already changed. The cells become able to form tumors in the lab because they grow and multiply too much, can live forever, and don't need to stick to surfaces.

Cell preservation is the process of taking out, preparing and keeping cells for later use in research, science, or medicine. Cell preservation techniques try to keep cells healthy by giving them the right environment to keep their special characteristics. It is hard to achieve because cell cultures can easily get contaminated and then they can't be used. Moreover, preservation methods use extremely high or low temperatures to start the process of protein denaturation in cells, which causes the cells to die. Therefore, it is important to use the right method to preserve cells and keep them from dying. Different methods are used to preserve cells depending on what kind they are and how old they are. There are two main ways to keep cells from going bad freezing them or keeping them at a very low temperature. All the other ways to keep cells safe come from these two main ways [5], [6]. Keeping organs cold only works for a few hours in a hospital if they will be transplanted soon. But freezing them can keep them good for a long time, even up to years. In the next part, we talk about some common ways to keep cells preserved. Hypothermic preservation is not mainly used to preserve cells. It is better for preserving tissues and organs, and for transporting them a short distance. Keeping cells in a cold state has good things like slowing down their activity and needing less oxygen. But it can also damage proteins. To prevent damage to cells from being too cold, we use special liquids with sugar and other substances in them [7], [8]. These liquids help cells stay healthy and prevent them from swelling or getting too acidic.

Viral transformation is when cells change and grow differently because they have been given new, inheritable material. This process happens when a virus changes a cell or group of cells in a harmful way. The term can also mean putting DNA into cells using a virus. Viral changes can happen on their own or through treatment. Natural transformations can be caused by viruses like human papillomavirus (HPV) and T-cell Leukemia virus type I, which can lead to cancer. Hepatitis B and C are caused by viruses changing the cells in the body. Viral change can be created to use in medical treatments. We can tell the difference between cells that have been changed by a virus and those that haven't by looking at how they grow, their surface, and what's inside them. Changes in the way cells grow can happen because they are no longer limited by contact with other cells, they grow in different directions, and there are too many cells in a small space [9], [10]. Cells change and may lose their connections, speed up nutrient transfer, and produce more protease. Change can also impact the cell's structure and the amount of signaling molecules.

There are three different kinds of viral infections that can be thought about in the topic of viral transformation. These infections can kill cells, last a long time, and change the cells. Cytocidal infections can make cells stick together, stop things from moving through the cells, mess up the cell's DNA, RNA and protein making, and usually cause cells to die. Long-lasting infections happen when a virus stays inside a cell without being active until something makes it start working again. This infection doesn't always make big changes in the cell, but it can cause ongoing illnesses. Changing infections can also be called cancerous transformation. This infection makes a cell change and become cancerous. It has the potential to either destroy the cell commonly seen with RNA viruses or remain within the cell for an extended period often observed with DNA viruses. Cells that have been infected and changed can last forever and pass on the genes that cause tumors to grow. Because the word cytocidal means cell death, these three infections can happen at the same time. Many cancer-causing viruses that change how cells grow and multiply also end up killing the cells.

DISCUSSION

Deadly infections often cause changes in the way cells look and work. This is important for the virus to make more copies of itself and take over the cells. The effects of cytopathic include changes in the shape and structure of cells, like when cells fuse together to form larger ones and when they make special parts in their nucleus and cytoplasm. Changes in the body include not enough movement of ions, making secondary messengers, and starting a series of events in cells to keep them working. Many viruses stop the body's cells from making DNA, RNA, and proteins. They can also stop the cells from communicating with each other at a small level. Genotoxicity is when something causes damage to the chromosomes in a living thing, like breaking them or changing their structure. Finally, the viruses can affect the way the immune system works in the body. Two kinds of infections can kill cells in the body, called productive and abortive. During active infections, additional viruses are produced.Abortive infections do not create new viruses that can spread to other people. One type of virus that kills cells is the herpes virus.Three kinds of infections stick around for a long time [11], [12]. The virus stays inside the host's cell for a long time. During latent infections, the virus doesn't show much signs of being active in the body. The virus's genetic material remains dormant within the host cell until it is needed for replication.Long-lasting infections have the same effects on cells as severe infections, but they produce fewer new cells and viruses that can change cells.

Finally, slow infections take longer to show any physical, structural, or cellular changes in the body. Transformation infection only happens during failed or limited infections. This category of infections is the most widespread due to its ability to encompass both cell-killing and long-lasting infections.Viral transformation mostly means changing infections, so the rest of the article talks about how infections change. For a virus to change a cell, the virus's DNA needs to get inside the cell. The easiest thing to think about is when a virus changes a bacterial cell. This process is called lysogeny. This process is called lysogeny. The virus DNA can either stay in the body without causing harm until it is triggered by something like UV light, or it can quickly become part of the host's DNA. In both cases, the virus DNA will make copies of itself when the cell divides, infecting two cells. The process will keep spreading more and more infected cells. This process is different from the lytic cycle [13], [14]. During the lytic cycle, a virus takes over the host cell's replication machinery to produce more of itself and ultimately causes the host cell to burst.The same thing happens in animal cells too. Usually, instead of putting viral DNA into an animal cell, the virus is covered by a part of the cell's outer layer. Then, the cell takes in both the virus and the covered part of the outer layer.

Viral transformation changes how the host cell's genes work. instead of working as they should, the cell's genes start to work like the virus genes. The virus can also stop cells from talking to each other and make them multiply faster. Viral change can give a cell specific trait. Typical physical changes consist of higher cell density, unanchored growth, continuous growth in the presence of other cells, loss of growth control, immortality, and structural cell disruption. Viral genes are made using the host cell's machinery. Therefore, numerous viral genes possess promoters that facilitate their binding to the transcription factors present in the host cells.Both the virus and host cell's genes can be controlled by these proteins. Many viruses can make the cell produce more regulatory proteins. Depending on the virus, the host cell can undergo different genetic changes. In the lytic cycle, the cell only lives long enough for the virus to make more copies of itself. Sometimes, the germ's DNA stays in the host cell and makes copies of itself when the cell does. This viral DNA can be added to the host cell's genetic material or remain as a separate genetic form. Both situations can harm the chromosomes of the host cell [15], [16]. The damage might be fixable. But usually, the genetic material becomes unstable or the gene expression is changed.

An assay is a test used in a laboratory to measure the quality of something. In virology, assays can help tell the difference between changed and unchanged cells. Using different testing methods can change how the cells are affected and what traits are chosen in the changed cells. Three popular tests that are used are the focus forming test, the test for growth without being anchored, and the test with less serum. The focus forming assay (FFA) is a test used to grow cells with a cancer-causing gene on a layer of healthy cells. The changed cells will create lumps on the sample as they grow, and this test is very sensitive for studying viruses. An example of a test for cell growth that doesn't depend on being attached to a surface is the soft agar assay. The test is checking how well the cells can grow in a thick liquid. Cells that have changed can grow in this place and don't need to be attached to anything. Cells that need to be attached to a solid surface in order to grow are called anchorage dependent untransformed cells. This test is considered very strict at finding cancer. In a simpler test, cells are checked by seeing how they respond to changes in the stuff they need to grow. Regular cells need at least 5% serum to grow, but changed cells can grow with less serum.

Without any assistance from medicine, cells can become infected by a virus through natural transformation. The most frequent method by which a virus can alter your cells and lead to illnesses such as HIV, Hepatitis B, and T-cell Leukemia virus type I. Viruses can cause about 20% of human tumors. Viruses such as HPV, T-cell Leukemia virus type I, and hepatitis B have the potential to cause tumors. Cancer caused by viruses is most often seen with DNA and RNA tumor viruses, especially retroviruses. There are two kinds of cancer-causing retroviruses: ones that transform cells quickly and ones that do not. Fast-growing viruses can make tumors grow quickly because they have viral genes that cause cancer. One example of a strong changing virus is the Rous Sarcoma Virus (RSV) which has an oncogene called v-src. v-Src is a protein that helps cells grow really fast. On the other hand, a non-acute virus causes tumors to grow slowly because it does not have any cancer-causing genes. It makes tumors grow by turning on certain genes called proto-oncogenes.

Cancer caused by a virus can happen in two ways. The tumor virus can put a gene into a cell's DNA that changes the cell, making it grow out of control. The virus can change how the genes of the host are used. One or both of these actions can happen in the same cell. The Hepatitis B virus protein X is thought to cause liver cancer by changing liver cells. The virus's DNA is put into the host cell's genetic material, which makes the cell grow and divide quickly, creating a tumor. Papillomaviruses usually infect skin and mucous membrane cells

and can cause different types of growths, including warts and cervical cancer. When HPV changes a cell, it messes up some proteins and breaks down others. The herpesviruses called Kaposi's sarcoma-associated herpesvirus and Epstein-Barr virus can cause cancer in people, like Kaposi's sarcoma, Burkitt's lymphoma, and nasopharyngeal carcinoma. Even though scientists have found some genes in these viruses that can change the cells, they still don't know exactly how the virus does this or how it makes the host cell copy itself. Retroviruses are a type of virus that includes T-cell Leukemia virus type I, HIV, and Rous Sarcoma Virus (RSV). The viral gene tax is turned on when the T-cell Leukemia virus changes a cell, making it grow uncontrollably and become cancerous. HIV doesn't make cells cancerous, but it makes infected people more likely to get lymphoma and Kaposi's sarcoma. Many other retroviruses have three genes called gag, pol, and env. These genes do not directly cause the growth of tumors or transformation in cells.

The human immunodeficiency virus is a virus that attacks the body's lymph nodes. HIV attaches to the immune CD4 cell and then changes the cell's genetic material with the help of reverse transcriptase. This allows the virus to become a part of the cell's DNA using integrase. The virus makes copies of itself using the host cell's tools and then goes out of the cell to infect more cells. Viral transformation can be made to happen in a cell culture for treating sickness or other conditions in many ways. Cells in a dish are infected with a virus, which changes them. These changed cells can then be used to make medicine or put directly into the body. Type I interferons (IFNs) are a kind of medicine that is used to treat many different health problems like hepatitis C, cancer, viruses, and inflammation. IFNs can come from either natural source like human cells or blood cells, or they can be made using genetic technology. Many of these treatments for IFN have a small chance of working. The Epstein-Barr virus (EBV) can be used to make personalized IFNs. During this process, special white blood cells called B lymphocytes are changed with EBV.

These cells can be used to make IFNs that are specific for the patient from which the B lymphocytes were taken. Making the treatment personalized reduces the chance of the body making antibodies against it, making the treatment work better. When a virus changes a cell, it can make the cell do things that lead to cancer. This can happen by changing the cell's genetic material or adding more genetic material, which makes the cells grow out of control. Some people don't realize that the virus causing cancer can also be used to slow down or even stop the cancer from growing. Viruses change host cells to survive and make more of themselves. But the immune responses of the host cell are weakened during this change, making the cells more likely to be infected by other viruses. In 1951, people first thought about using viruses to help treat cancer. They had this idea because a 4-year-old boy with leukemia got better for a short time when he had chickenpox. This led to scientists doing research in the 1990s. They wanted to make a type of herpes virus that could infect and change cancer cells, but not hurt healthy cells. Treating patients with viral therapy may be safer and better than using chemotherapy. Viruses that are used to treat cancer get stronger and work better as they grow in the body. They only cause some small side effects like nausea, tiredness, and aches.

CONCLUSION

Cancer cells don't follow the rules of normal cell growth and can keep growing and piling up. Regular cells stop growing and die after about 50 generations, but changed cells can keep growing and be grown forever. Altered cells no longer have the same requirements for growth factors as normal cells and often require less nutrients. Transform cells can grow on agar in clusters, whereas regular cells require contact with a specific surface to grow. Cancer cells grow in special mice that have weak immune systems or genes that affect their ability to

fight off diseases. These mice are unable to facilitate the development of healthy cells from other animals. Transformed cells have different numbers of chromosomes than normal cells. Typical cells contain their standard number of chromosomes. Cancer cells can have different markers on their surface, known as tumor-associated antigens. These markers are usually caused by the virus genetic material being activated in the cells. Certain proteins found on the surface of cancer cells can be targeted by the immune system. These proteins are called tumor-specific transplantation antigens. When the immune system doesn't get rid of cells with tumor-related proteins on their surface, it can lead to the development of certain cancers.

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CHAPTER 7

TRANSGENIC ANIMALS: PRODUCTION PROCESS, AND APPLICATION

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ABSTRACT:

Animals that have undergone genetic modification have had their genes altered to fulfill diverse functions, such as manufacturing medication, boosting crop production, and maintaining wellness. Most genetically modified animals are still being studied in labs, and only a few are close to being sold to the public. Through the use of science, mammals' genes can be modified to create genetically engineered animals. They are a special type of organisms that have been changed using genetic technology. The majority of studies involving genetically modified mammals focus on mice due to the difficulty in creating knockout animals in other mammal species. This is because we can't grow their embryonic stem cells well. A transgene is a gene that has been relocated from one organism to another, through either natural processes or genetic manipulation. Adding a new gene to an organism could change how it looks or acts. This is called transgenesis. Transgene means a piece of DNA that has a gene from one living thing and is put into another living thing. This foreign piece of DNA can either make the transgenic organism produce RNA or protein, or change how the organism's genes normally work. Usually, the DNA becomes part of an organism's reproductive cells. For instance, in larger animals, the foreign DNA can be put into the center of a fertilized egg. This technique is frequently utilized to introduce human disease genes or other significant genes into laboratory mice. This helps scientists understand how the gene works and what problems it might cause.

KEYWORDS:

Foreign DNA, Gene, Genetically Modified, Stem Cells, Transgenic Animals.

INTRODUCTION

Transgenic animals are commonly used in labs to study medical research. More than 95 percent of the animals used are modified mice. They are very useful for studying human diseases. Scientists use them to understand how genes are involved in disease, how the disease gets worse over time, and how the body responds to treatments. Mice have been changed in their genes so they can make human antibodies. These antibodies can be used as treatments for illnesses. Between the years 2006 and 2011, seven out of eleven FDA-approved drugs made from antibodies were produced using genetically modified mice. Scientists are looking into using genetically modified farm animals to make a lot of important human proteins that can help treat diseases. Right now, special proteins used for treatment are made in reactors using mammal cells, but it costs a lot of money. In 2008, a new factory that made a kind of medicine cost more than $500 million to build. A less expensive choice would be to find a way to make proteins using transgenic animals' milk, blood, or eggs. There has been little progress in this area so far. Only two medical products have been given the okay by the government so far [1], [2]. The first thing is a medicine called antithrombin III that is

made in the milk of special goats. It helps stop blood from clotting in people with a certain condition when they have surgery or have a baby. A group of about 80 goats can provide enough antithrombin III for all of Europe. The second product is a medicine that prevents C12 esterase in humans. It's made in the milk of special rabbits. This is medicine for a rare genetic disorder that makes blood vessels expand and causes swelling on the skin [3], [4].

The ability to create transgenic animals depends on several factors. One of the first things we need to make transgenic animals is the ability to move embryos from one animal to another. In 1891, Walter Heape successfully moved embryos from one Angora rabbit to another. Another important part is being able to change the embryo. In the 1940s, scientists first started making changes to embryos in mice outside of the animal's body using a special growing method. It's also important to be able to handle eggs. Ralph Brinster from the University of Pennsylvania figured out how to grow eggs in 1963, and Teh Ping Lin from the California School of Medicine developed a way to inject DNA into fertilized mouse eggs in 1966. The first time animals were changed genetically was in 1974. It was done by two scientists named Rudolph Jaenisch and Beatrice Mintz. Rudolph worked at the Salk Institute and Beatrice worked at the Fox Chase Cancer Center. They showed that it's possible to change genes in mice by injecting a virus into young mouse embryos. The mice that were born had the changed gene in all parts of their bodies [5], [6]. In 1976, Jaenisch found that the Moloney Murine Leukemia Virus could be passed from parent to baby by infecting an embryo. In 1980, Jon Gordon and George Scango, with Frank Ruddle, said they made a mouse with added genetic material. This mouse was born four years after they started working on it. By 1981, other scientists had put different DNA into mice and changed how the animals were made. This work helped make mice that have been changed to get certain types of cancer. These mice were created in a lab to help researchers learn more about how cancer starts and develops. These mice are good because they are very similar to humans. Mice help us learn more about cancer and also test new drugs [7], [8].

Scientists purposely insert genes from other organisms into the DNA of animals, like mice, to create transgenic animals. Typically, these creatures are created by inserting genetic material into a fertilized egg, which is then placed into a surrogate mother for gestation. This means the animal that gets the modified genes will have babies with the changes too. The babies are then mated with other babies that have been genetically modified to create a new line of genetically modified offspring. We can make transgenic animals by putting DNA into special cells and then putting those cells into a very young embryo. We can also use viruses to put the DNA into the embryo. This last way is often used to change one gene. Usually, this means taking out or turning off a specific gene. The outcome is called a "knockout" animal. Since the 1980s, scientists have been using genetically modified mice to study disease. Mice are used as a model because we know a lot about their genes and their genes are like ours [9], [10]. Furthermore, tests done on mice, such as their physical and behavioral reactions, can be used to understand human diseases. Simple and advanced methods are also easy to get for changing mouse cells and embryos. Another good thing about mice is that they have babies often. Other genetically modified animals like pigs, sheep and rats are also used in research. But they are not used much in medicine research because of technical problems. New technology is making it easier for more people to use transgenic rats.

Modified mice and rats are really important in finding and making new drugs. They help scientists study how genes work in the whole body, which has improved our understanding of how the body works and diseases. It has also helped find new targets for drugs. Because humans and rodents have similar bodies and gene functions, scientists can make transgenic rodents that have the same diseases as humans. Certainly, many different mice with modified

genes have been created for this goal. Mice are being used as examples to study different health problems like obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging, Alzheimer's disease, and Parkinson's disease. They are also used to learn about different types of cancer. Also, scientists are studying transgenic pigs to see if their organs can be used for transplants. If this is safe for patients, it could help with the shortage of donor organs. Transgenic animals are being made faster and cheaper now because of a new gene-editing tool called CRISPR. It has made the process much simpler and quicker.

DISCUSSION

Transgenesis involves putting new DNA into cells and making sure it becomes part of their genes and gets passed on to the next generation. Transgenesis in animals can help increase the number of babies they have and how well they reproduce. It can also help them use their food better and grow faster. Transgenesis can also improve the quality of the meat and milk they produce, and help them fight off diseases. The growth hormone gene plays a crucial role in promoting faster growth and increased milk production in farm animals. In germ-line gene transfer, changes are made to the parents' egg and sperm cells so that the offspring of the changed species will also have these changes. Today, many food animals like cows, sheep, goats, pigs, rabbits, chickens, and fish have been given gene constructs. A big success in farming is when a gene is added to an animal or plant's genetic code in a way that it will be passed on to future generations. Animals with big genes are useful for studying genetics and biotechnology. They can help us understand and control genes that are responsible for multiple traits. Different ways to create transgenic animals have been made in the past few decades.

Many genetic sequences have been found by studying genes, which helps us understand the important parts of DNA for different species. The use of new technologies in genetics and reproduction shows that we can create transgenic animals successfully. The way to make a transgenic animal depends on how it will be used. Several genetically modified animals have been created for studying how genes work, making biological products, and testing new ways to breed animals. The main methods used to make transgenic animals are shown in the picture. The sentence is missing, can you please provide the text that needs to be rewritten. There are three ways to move foreign DNA into an organism: putting it into an early cell, using sperm or egg cells, or using a special method called somatic cell nuclear transfer. A cloning vector is a small piece of DNA that can copy itself and be used to transfer or spread foreign DNA in an organism. Vectors make it more likely that genes will be used by the cell [11], [12]. Different types of vectors have been created to store DNA of different sizes. Plasmids, cosmids, P1 phage, BACs, and YACs can hold different amounts of DNA. Plasmids hold 20 kilobytes (kb), cosmids hold 40 kb, P1 phage holds 90 kb, BACs hold 200 kb, and YACs hold 1000 kb of DNA. Viruses can easily put their genetic material into cells. Scientists were interested in using viral DNA as a way to deliver new genes.

RNA viruses can make DNA from RNA using reverse-transcriptase enzymes. When a cell divides, the virus can make copies of itself by becoming part of the host cell's DNA. Lately, scientists used retroviral vectors to put a new gene into the host's genes. They can hold around 7 to 8 kb of genes from other places, but it might not be enough for really long genes or structures that need a lot of control sequences for making proteins. Adeno-associated virus (AAV) was first found in human tissues in the 1960s from laboratory adenovirus (AdV) preparations. Some research teams studied AAV biology just because they were curious and didn't realize its huge potential for helping people with gene therapy. In the first 15-20 years of research, scientists discovered some important things about the virus. They learned about its genetic material, how it multiplies, how it can stay inactive in the body, and how it puts

together new virus particles. The wild-type AAV2 sequence was successfully cloned into plasmids, which allowed for genetic studies. The full AAV2 genome was also sequenced because of these achievements. These first studies gave important information that helped create AAV as a way to carry genes, which could hold about 10 kb of DNA from outside sources.

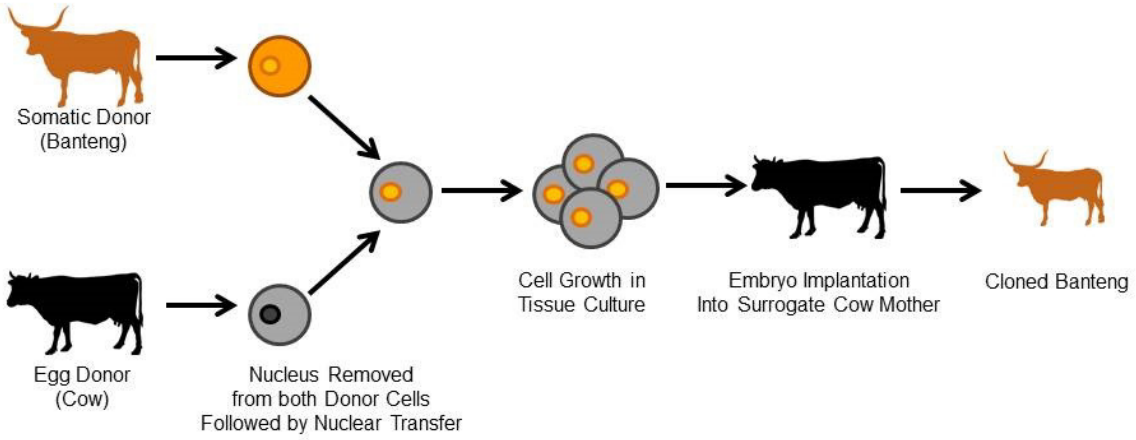

Figure 1: Representing the overview about the somatic cell nuclear transfer [WildlifeSNPits-wordPress].

Adenoviral vectors are a type of DNA that is not covered by an outer layer. Adenoviral vectors are commonly used in laboratories and in small animals for research because they are easy to make and can express genes at high levels. Adenovirus vectors are very powerful tools for moving genes, and they can hold up to 10 kb of foreign DNA. The retrovirus often removes certain genes that help it make more virus particles. This is a common change in this type of virus. Today, transgenic technologies are very important in genetics. They help scientists study and change gene function in whole animals. In farming, scientists change the genes of animals to make them better at producing things like milk, wool, and being healthy. In biotechnology, scientists are trying to use big genetically modified animals, called bioreactors. To make expensive medicines at a low cost by using special sheep or cows that were modified. Transgenic technology can also be used in xenotransplantation [13], [14]. In this field, pigs are genetically modified so that their organs can be transplanted into humans without being rejected by the immune system. So far, transgenic technology has mostly been used for basic research, studying how genes work and how diseases develop. Putting the genes that make toxins into certain cells in mice has been a useful way to study how mammals grow and how cells are related to each other. Changing or interfering with the way genes work within the body by adding new genes has been used to find and copy important genes involved in development. Using animals to study human diseases has been greatly influenced by transgenic technology. Research in these areas has used apps to study how diseases work, the effects of treatment over a long time, and the harmful side effects of gene therapy. It has also looked at how genes and the environment work together to cause illness.

The creation of transgenic animals started by transferring genes with sperm in the 1980s. Transgenic mice were made using a microinjection method. After that, scientists said they made many animals with changed genes in 1985. There are different kinds of modified animals, such as chickens, sheep, pigs, birds, and insects. Microinjection was a popular method used for making transgenic organisms, but it has problems like not being very effective and not always working the same way. So, scientists started to search for different ways to put foreign DNA into cells, like putting sperm heads with DNA into cells, using

viruses, using RNA interference, or using nuclear transfer. Transgenics is when scientists take genes from one type of plant or animal and put them into a different type of plant or animal. A transgenic organism is one that has been created by putting a gene into it using scientific methods in a lab, instead of through traditional breeding.

The first thing we do is find the gene we want that makes a certain protein. First, scientists need to find the gene that makes a good trait or protein. There are ways to find the specific gene using techniques like Gene Chips and DNA sequencing. The next step is to separate the gene we want from the species we are studying. It can be done by either using force to break the cells or using chemicals like soap to help break them (Figure 1). The DNA is removed from the other parts of the cell using a method called cell centrifugation. First, the target gene needs to be separated from all the other DNA. This is done by separating the DNA fragments based on their size using a technique called Gel Electrophoresis. The gel is cut and copied using PCR. Alternatively, the gene we're interested in could be put into a small piece of DNA inside a bacterium using a special enzyme called DNA Ligase. Bacteria can copy genes when they divide, which is called Gene Cloning. If we have enough information about the gene we are interested in, we can make special DNA primers and copy the gene using PCR without needing to isolate it on a gel. At the end, a vector is used to put the gene into the organism that is being changed. The final DNA sequence, which includes the gene we want and its control sequences, is called Gene Construct [15], [16]. However, only a small percentage of transgenes are successfully activated. The gene needs to get into the nucleus in order to be turned on. In order to be passed on when cells divide, it needs to combine with the target cell's genes using recombination/crossing over during mitosis and meiosis. To check if the desired gene has been added to the genome, scientists put in another gene called a reporter gene with it. This gene controls traits that are easy to see or measure, such as being able to resist antibiotics or produce a glow-in-the-dark protein. This helps the scientists check if the gene is working or not. There are many ethical and legal issues connected to genetically modified animals. Transgenic animals are used a lot to study diseases. Genetically modified animals used for eating.

Transgenic animals are produced through the insertion of a new gene or genes into their bodies. The new gene from another organism is called the transgene. The DNA Microinjection method is when a gene is injected into a fertilized egg from the same species or a different one to change its characteristics. Microinjection is a common and widely accepted method in mammals because it can be used on many different types of animals. However, the new gene from another organism might end up being too active or not active enough, depending on where it gets put into the DNA. This process is not very predictable. The modified fertilized egg is placed into the oviduct of a female animal that has been prepared to receive it by mating with a male who can't make babies. Stem cells are special cells that can turn into different types of cells in the body, like skin cells or egg and sperm cells. They can also help make a whole new living thing. Embryonic Stem Cells (ESCs) come from the beginning of an embryo's development. from mouse or human pre-embryos. They are versatile cells that can make copies of themselves. They can be kept in culture for a long time as cells that have not changed or specialized. These cells can be encouraged to change into any type of cell. ESCs can turn into different parts of a growing embryo or any kind of body cells. This is an important method for fixing tissues. It includes putting the DNA we want into stem cells in a lab. These cells are grown in a special liquid and put into an embryo when it is still in the early stage of development.

This makes a mixed-up animal. The best way to turn off a gene is by using ES cells and gene transfer, also known as the knock-out method. This method is very important for studying

how genes control how living things grow and change. This way of working has the benefit of being able to target specific changes in the gene using homologous recombination. Retrovirus brings in new genes using a carrier, usually a virus or a plasmid. Retroviruses are often used to carry genetic material into cells because they can easily infect the cells. This helps in transferring DNA or RNA into the cells. Animals born from this process are mixed, which means that not all of them have the retrovirus. The new gene can only be passed on if the retrovirus becomes part of the germ cells. Below, we talk about the steps of the technique: First, the center of a body cell is moved into the cytoplasm of an egg that has had its own center removed. Secondly, the center of the cell in the egg changes to become a fertilized egg because of factors in the egg. Thirdly, the zygote is made to split into two cells by an electric shock. Finally, when the tiny baby grows into a ball of cells, it is placed in the belly of a woman who is carrying it for someone else. Transgenic animals are commonly used to study diseases. In the past, mice have been used because their bodies are similar to humans' bodies. Researchers like to use special mice with changed genes to study serious diseases like Alzheimer's, cancer, and AIDS.

Transgenic animals help scientists understand how genes are involved in certain diseases. The FDA says it's okay to eat animals and products from cloned animals. Transgenic animals are often used to make helpful proteins for medicines that treat diseases like emphysema or cystic fibrosis. Companies are spending a lot of money to study how to make medicine from the milk of genetically modified animals like rabbits, goats, and cows. They want to use this medicine to treat diseases like cancer, rheumatoid arthritis, and other autoimmune disorders. Researchers in Australia changed the genes of the mouse pox virus to create new mice. Xenotransplantation is when living tissues or organs from one animal are put into another animal. This could help with the problem of not having enough human organs like hearts and kidneys for transplants. Pigs have similar body parts and organs to humans, so they are good for transplants into humans. Scientists are also finding out how putting cells into the body can help people with serious diseases like Parkinson's or spinal cord damage. Using genetic changes to control stem cells, we can grow tissues on a framework to make temporary skin for healing cuts or burns. Tissue engineering is being used more and more to replace parts of the human body, like shunts, heart valves, cartilage, and other organs. Mixing genes from different organisms can help scientists make vaccines for dangerous sicknesses. For example, scientists put human tumor DNA into tobacco plants to make a vaccine for non-Hodgkin's lymphoma. Similarly, scientists have made a flu shot using human DNA and tobacco plants. Transgenic pigs are used to make human blood. Special animals are used to test if chemicals are harmful. Genetically modified animals are used to make milk. In general, genetically modified mice can help make milk better. In the same way, scientists use genetically modified pigs to make more milk by changing the lactose in it. Also, genetically modified sheep are used to make wool. Scientists everywhere are working hard to create animals that are resistant to diseases like the flu, for example pigs that don't get sick from the flu.

Genetically modified mice, rabbits, pigs, sheep, cows, chickens, and fish have been made by combining their sperm with DNA from another organism and then fertilizing the eggs. In addition, you don't need any special equipment or skills to do this, and you can do it outside. Another interesting thing about using sperm as DNA carriers is the idea of mass genetic modification. In later studies, they were able to put a special gene into the sperm of sheep to make them grow faster. This was done to help produce more meat in Egypt. The place where foreign DNA attaches to mouse sperm is controlled by a group of molecules called class 2 major histocompatibility complex. In the fluid of a male mouse's reproductive system, scientists found two things: a substance that breaks up DNA from the seminal vesicle and different proteins that stick to other kinds of DNA from the prostate. The things in the cells

can stop the outside DNA from getting stuck. SMGT is used in farm animals like cows and pigs. It takes advantage of the farmers' usual method of artificial insemination. We take fresh semen from donor animals and clean it many times before spinning it to remove the liquid part. Animal artificial insemination means taking sperm from one animal and putting it into another animal to help them have babies. Scientists also tried putting sperm in a mixture with DNA, then adding some chemicals to help the DNA get into the sperm. This made it easier for the DNA to get into the sperm. The protective layer around the sperm was weakened, which let other DNA enter the sperm easily. Additionally, similar results have been seen using sperm freezing and thawing.

Another interesting method is intracytoplasmic sperm injection, where sperm that has been treated with foreign DNA is injected directly into the egg. ICSI has helped move long DNA pieces in mice, yeast, bacteria, and other man-made chromosomal constructs. A fascinating way to create genetically modified animals by mixing sperm cells with marked foreign DNA and monoclonal antibodies. mAb C is a basic protein that sticks to DNA using electrical interactions, which helps to attach foreign DNA to sperm in a selective way. The outer part of the sperm from different animals, like pigs, mice, chickens, cows, goats, sheep, and humans, reacts to this linker protein. It is important to remember that the way organisms take in foreign DNA is a significant part of their biology. The process of making mature sperm from stem cells happens at different times as the cells change and grow. Sperm stem cells can be taken out, grown in a laboratory for a short time, and then put into a different testis. The moved cells keep changing and eventually make working sperm. Giving male recipients busulfan, a medicine that stops testis stem cell growth, greatly increased the number of sperm made by the transplanted stem cells. This method has been used to put genes into stem cells while they are being grown. This was done using a strong virus. Transgenic mice were made at a speed of up to 4% when stem cells were put into busulfan-treated males.

This technology can be used to study how genes affect the development of sperm cells and to make animals with modified genes. It's unlikely that we will be able to predict results for bigger animals than mice. Certainly, we need more changed cells in order to increase the likelihood of spreading to the testicles quickly. Other ways of making genetically modified sperm cells have been looked into. One way to transfer genes is by using testis. It's like SMGT but simpler because it doesn't use IVF or embryo transfer. Also, the testis is considered to be an organ that is protected from the immune system. Being able to put genes into certain testicular cells while they're inside the body could help us study how sperm is made. Scientists are studying how to put new genes into sperm cells in the testicles. Foreign DNA inserted into the testis can quickly move to the epididymal ducts and be taken in by cells and sperm in the epididymis. A solution containing a type of virus called adenovirus is injected into the testicles of a mouse as a way to add new genes. This is done by injecting the solution into the space between cells or into the tubes inside the testicles. The study shows that using a virus to transfer genes could be a good way to change the cells in the testicles. This could help with male infertility in the future, even though it might cause some problems with sperm production and an inflammatory response. The results also show that TMGT could be used to treat gene problems in babies before they are born and to make genetically modified animals.

Creating animals through nuclear transfer is not very effective, and the success rate is only 0. 5 to 50% Problems can happen during pregnancy, when the baby is born, and in the weeks and months after. Some babies may have developmental issues. We don't know what causes these abnormalities, but they could be due to mistakes in the way genes are programmed or problems with certain genes. We might be able to understand how these processes work

better if we have a good understanding of the systems that control normal growth. The method involves moving a cell's nucleus into an egg cell without a nucleus. The cytoplasm of the egg cell modifies the nucleus, resulting in the formation of a zygote. In animals like mammals, the fertilized egg must be placed into the uterus of a different mother to grow. In 1986, Willadsen had success by cloning lambs from embryos that were 8 to 16 cells old. This finding made researchers interested in using nuclear transfer to make more embryos from important farm animals. This long process also brought new and exciting possibilities for creating animals with different genes. When the cells used to make embryos have genetic changes, the animals made from them may be considered transgenic animals because they have added, changed, or replaced genes. This view posits that transgenic embryos and animals are produced by transferring genetically modified cells into another cell to create the animal.

CONCLUSION

Introducing genes from different species in transgenic animals could potentially improve the quality of life for humans. Transgenic animals are created using DNA microinjection, stem cells, viruses, and artificial chromosomes.Transgenic technology offers significant promise in diverse sectors, such as farming, healthcare, and manufacturing. The public will decide if genome editing is okay based on how it helps people and if it's fair. They will be more accepting of genetic alterations in animals if it improves their well-being. The public likes apps that help animals. Using genome editing to create transgenic animals has helped make animals healthier and happier, and has improved how much they produce and the quality of what they produce. This benefits the increasing number of people around the world.

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CHAPTER 8

FETAL CELLS FOR TRANSGENIC ANIMALS: UNVEILING THE DEVELOPMENT AND POTENTIAL OF TRANSGENIC ANIMALS IN BIOTECHNOLOGY

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ABSTRACT:

In transgenics, genes are transferred from one plant or animal to another. A transgenic organism is an organism that has had a gene added to it in a lab, rather than through natural breeding. - The technique of creating transgenic animals commenced in the 1980s by transferring genes through sperm. The most common way to make transgenic mice is by using microinjection. After that, scientists made many transgenic animals in 1985. There are different kinds of genetically modified animals, such as chickens, sheep, pigs, birds, and insects. Microinjection was a popular way to create transgenic organisms, but it has some big problems like not working very well and not always giving the results we want. So, scientists started to search for different ways to add foreign DNA to cells, such as injecting sperm with foreign DNA, using viruses, RNA interference, and nuclear transfer technology. Transgenic animals have many ethical and legal issues. Transgenic animals are used in different ways. They can help researchers understand and find treatments for diseases. They can also be used as food and in drug and industrial production. Additionally, they can be used to control diseases and in xenotransplantation. They may also be used to develop vaccines and help with blood replacement. They are also used in testing for toxicity and in agriculture. In this unit, you will learn about animals that have been genetically modified using cells from unborn babies, as well as animals that have been genetically modified for organ transplant purposes. You will also learn about using genetically modified organisms to stop the spread of diseases, as well as artificial insemination and embryo transfer technology.

KEYWORDS:

Cells, Egg Cell, Gene, Nuclear Transfer, Stem Cells.

INTRODUCTION

Stem cells are special cells that can divide and change into different kinds of cells based on what the body needs. Stem cells have special qualities. They are cells that are not specialized. They can make copies of themselves by dividing into two different cells. Answering this question is not easy. They can transform into various cell types based on signals they receive and the body's requirements. Fetal stem cells have special features: They are found in the organs of developing babies. They can turn into two types of stem cells: pluripotent stem cells and hematopoietic stem cells. Cells from developing babies, such as fibroblasts, can turn into any type of cell. Researchers were successful in making copies of cells from embryos to create new sheep and bull calf with special genes. In this process, foreign genes can be put into cells of developing animals to make them produce useful items. Cells from a 55-day-old cow fetus were collected. These cells are very special and can grow in a special mix of nutrients. The desired trait can be inserted into fibroblasts using a small injection or another

suitable method. The nucleus of a fibroblast cell with changed DNA is taken out of the cell [1], [2]. Then it is placed into a cow egg that has had its nucleus removed. The sentence is incomplete and lacks context, could you provide more information.

This egg cell is divided to form a blastocyst in a liquid. Then, it is put into another mother cow to make transgenic baby cows. Organ transplants like liver, kidney, and heart surgeries are now very advanced. They are capable of swapping out nonfunctional organs for functional ones. As the need for human organs increases, there are not enough donors to give them, which is a big problem. This has caused patients who need organs to die because they had to wait too long. Xenotransplantation is when living tissues or organs from one animal are put into another animal. This could help because there are not enough human organs like hearts and kidneys to go around. Pigs are good for organ transplants because their bodies are similar to ours [3], [4]. The person's immune system makes antibodies to attack the donated organ.

Activation of the body's defense system when receiving an organ transplant. Scientists are working hard to use pig organs instead of human organs that don't work well. They found out that the main reason why primates reject pig organs is because pigs have a special type of sugar that primates don't have. This sugar is made by a specific enzyme in pigs. Researchers are excited because a method called "knockout" could be really helpful. In a few years, scientists might be able to create pigs with a gene that makes a special enzyme. Another way to do this is by putting the gene that makes the enzyme á 1, 3 galactosyltransferases into primates. Scientists are hopeful that using this method will make the recipient's immune system react less. Using the method mentioned above, scientists are more likely to stop the body from quickly rejecting organs from other people. Next, the problem is that the host's cells are taking too long to reject the foreign organ. This involves cells called macrophages and natural killer cells. Another worry about xenotransplantation is that the pig retroviruses may become active after the organ is transplanted, causing unexpected changes in the patient's genes [5], [6]. Although the ideas seem good, using genetically modified animals for xenotransplantation is still being tested and it's unclear if it will actually happen soon. Also, the idea of using animal organs for transplants raises ethical concerns. As a result, both bioethicists and the general public oppose it.

Trans-genesis will help stop the spread of human diseases by stopping the parasite's life cycle. In the past few years, many tries have been made in this area, but very few have been successful. In the future, genetically modified organisms may help to control many diseases and take care of the environment. Next, we will talk about some animals, like transgenic snails. Schistosomiasis is a sickness caused by parasites called Schistosoma. It makes people sick with symptoms like fever, diarrhea, chills, and intestinal ulcers. The parasite gives people disease when it enters their body through the skin while they are in water. We can use genetically modified snails to stop the Schistosoma from reproducing. Some scientists have tried to create genetically modified snails that can stop a parasite from invading them. In the future, we may be able to stop Schistosoma's life cycle by releasing certain snails into the environment. Female Anopheles mosquitoes spread a dangerous disease called malaria [7], [8]. Scientists have found important genes that make the parasite spread to other organisms. In theory, it is possible to change these important genes to make a special kind of mosquito. Releasing a lot of special mosquitoes into the environment can help reduce the number of regular mosquitoes that spread malaria. This can help stop the spread of the disease. Bollworm is the name for the caterpillars that harm many different kinds of crops. Scientists made bollworms with a new gene that makes them die. When many of the special bollworms are let go outside, they will mix with regular bollworms and make babies.

These babies will not survive because they have a gene that makes them die. Many different types of plants can be saved by using this plan. Genetically modified Mediterranean fruit flies are causing damage to fruit and coffee crops globally. People are trying to use genetically modified medflies instead of wild ones to protect crops that people eat. 745 Genetically modified tsetse flies help spread African sleeping sickness, a disease caused by tiny organisms called protozoa. This illness affects the nervous system and can cause a person to go into a deep sleep that they can't wake up from. Scientists studying tsetse flies found a protein that can kill the germs that cause diseases. They made a special tsetse fly by putting a gene from another animal into the bacteria in its stomach. This way, we can stop the spread of illness.

DISCUSSION

In 1987, the inaugural Embryo Transfer Technology project in the country was launched by NDDB. They set up a central laboratory at Sabarmati Ashram Gaushala (SAG), Bidaj. The Department of Biotechnology (DBT), Ministry of Science & Technology, GoI gave money for the project to run for 5 years. from April 1987 to March 1992. From April 1987 to March 1992. NDDB started a project and set up one main lab and four smaller labs in different places in India. The main lab is in SAG Bidaj and the smaller labs are in CFSP&TI, Hessarghatta, ABC Salon, Shri Nashik PanchavatiPanjrapole in Nashik, and Buffalo Breeding Centre in Nekarikallu. NDDB helped set up 14 ET centres in different states of the country. Embryo Transfer (ET) is a technology used to help genetically superior female dairy animals have more babies. Normally, a very good female cow can have one baby calf in a year. With 'MOET/ET' technology, dairy workers can help a cow or buffalo have 10-20 calves in one year [9]. A really good cow/buffalo is given special hormones to make it produce a lot of eggs.

The FSH-like hormone makes the really good female produce lots of eggs instead of just one. The female animal is given extra hormones to make her produce more eggs. Then she is fertilized with sperm from a male animal two or three times, 12 hours apart, when she is ready to get pregnant. Seven days after being fertilized, the vets flush out her uterus to collect the growing embryos. Embryos are taken out with flushing liquid through a special filter. Then, the scientist looks at the quality of the growing embryos using a microscope. High-quality embryos are saved or frozen to use later. Or they are put into a different female animal about seven days after she is ready to have babies. "With ET technology, we can produce many high-quality calves each year. " SAG has done important work in this area and has made 14,388 viable embryos and 755 calves, which is the most by any organization in the country. Out of these, 1026 baby cows are from local cattle types, and 122 of them have been born. In addition, about 3000 baby buffaloes have been born [9], [10]. In 1991, the first buffalo calf in India was born from a frozen embryo as part of a project.

Somatic cell cloning is usually done by using cells that have been grown in a lab from small samples taken from the animal that will be cloned. The type of cell used is important for cloning to work well. Many types of cells have been used for cloning different animals, but people still can't decide which type works best for cloning. So, skin cells have been used most often for making copies of big animals because they are easy to get from live animals. When making transgenic animals through cloning, it's better to use cells from a fetus because they can grow in a lab for longer than cells from an adult. The way cells are grown in a lab can be different for each type of cell. For skin cells, a small piece of skin about 1-2 cm is removed after the area is cleaned with iodine and ethanol. The small piece of tissue is put in a special liquid with antibiotics and antifungal medicine and taken to the lab to be tested. In the lab, the sample is cleaned and the outer layer is taken off with a clean knife. The tissue that is

left is cut into small pieces (1-2 mm) and then put in culture. Instead, the tissue pieces can be broken down with collagenase (2-5 mg per milliliter) to separate the cells before they are grown in a lab. When using fetal cells, the fetal tissues are cut into small pieces with a knife and then broken down with a special solution. Cells are usually grown in a mixture of DMEM/F12, serum, and antibiotics. Cells can be kept in a lab for a long time or frozen and stored in really cold liquid. Even though some studies say that spending time in a lab doesn't seem to make cloning less effective, most cloned animals are made from cells that have only been kept in a lab for a short time [11], [12].

In many species, nuclear transfer is done by making the cell membranes of the donor cell and the egg cell fuse together. Fusion happens when electrical pulses are used with an electroporation machine. The cells and eggs that need to be fused together are put one at a time or in small groups (5-10) between the electrodes of the fusion chamber. The fusion chamber is connected to the electroporator. The way cells are fused together can change based on the type of cell, the animal they come from, the equipment used, and the liquid they are mixed in. Electrofusion usually uses an AC pulse to align cells in the fusion chamber and a DC pulse to make holes in the cell membrane and fuse the cells together. Usually, the AC pulse is around 5-6 volts at a frequency of 600-1000 kilohertz for 5-10 microseconds, and the DC pulses range from 1 to 3. 5 kilovolts per centimeter and from 30 to 250 microseconds. A solution that does not conduct electricity well is used to protect the cell-egg pairs from getting damaged when electrical pulses are applied. An often-used mixture for cloning animals includes a certain amount of mannitol, magnesium sulfate, calcium chloride, and BSA, with a pH level of 7.2-7.4.

In genetics and development science, somatic cell nuclear transfer (SCNT) is a way to make an embryo in a lab using a body cell and an egg cell. The method involves taking an egg cell with its nucleus removed and putting in a nucleus from another body cell. It is used for both medical and making copies of living things. In 1996, Dolly the sheep was known for being the first mammal to be successfully cloned. In January 2018, a group of scientists in Shanghai announced that they had successfully cloned two female crab-eating macaques named ZhongZhong and Hua Hua from fetal cells. "Therapeutic cloning" means using a method called SCNT in regenerative medicine. This method is seen as a solution to problems with using embryonic stem cells and destroying embryos for medical purposes. However, there are still questions about how similar these two cell types really are. Two ways have been created to move nuclei between young embryos. One is a surgery method that requires going through the plasma membranes of the cells. In animals with hair, it is harder and not as good at moving cell nuclei when it is done surgically, compared to a non-surgical method that uses cell fusion to help with the transfer. Before that, others had already figured out how to combine cells and were guessing about how it could be used [12], [13]. First, the cells are treated with cytochalasin, which stops the cells from making microfilaments. Then, they are treated with colchicine, which stops the cells from making microtubules. This helps the plasma membrane become more flexible.

This flexibility allows cells, or parts of cells, to be sucked into tiny tubes without breaking the cell walls. Even though these chemicals can be harmful, in baby mice, brief exposure does not cause any problems in their growth before they are born. After the cells are treated with these compounds, a small suction tool holds the outer layer of the embryo in place. Then, another small tool is used to carefully make a hole in the outer layer. The second pipette is moved next to the center of the cell where the chromosomes are. Sucking the stuff from the cell and taking out the pipette, then pinching the outer layer of the cell with the zona pellucida, takes out the nucleus. The nucleus can be moved from one egg to another inside

the outer layer of the egg. Instead, the zona pellucida can be divided with a glass needle, and then the contents can be moved from one zona pellucida to another using a third pipette. You can learn how to make small glass tools for these processes by reading Robl's book from 1988. When studying certain animal embryos, we may need to spin the eggs in a machine before we can see the nucleus inside. Spinning cattle and pig embryos shows the pronuclei and helps them keep growing. At this point, the karyoplast and the recipient cell need to be joined together. This happens when cells fuse together, which can be caused by an electrical pulse, Sendai virus, or polyethylene glycol [14], [15]. Both Sendai virus and electrical pulses can successfully combine mouse, rabbit, and sheep embryos.

Electricity has been used to help cells in cattle embryos join together. This method has only been reported for cattle embryos, but some viruses have also been tried. The shock of electricity needed to fuse the karyoplast and recipient cell can also wake up the egg cell. The way nuclear transfer is done in amphibians is like how it's done in mammals, but not exactly the same. Amphibian cells are sucked into a tiny tube called a micropipette. The tube is small enough to break the cell's outer membrane, but not break the cell completely. The inside of a cell is put into the middle of a developing egg at a specific stage of cell division. In mammals, when a cell nucleus is moved to another cell, a lot of cytoplasm is also transferred. This can cause some problems because the proteins in the cytoplasm can have bad effects. In some animals, the process of nuclear transfer starts the egg cell, while in other animals the egg cell needs to be activated separately. In amphibians, enucleation can be done by hand or with ultraviolet light. This is explained by Gurdon in 1986 and by Prather & First in 1989. In mammals, the egg cell is split in half and checked for certain chromosomes. If the half without the chromosomes is present, it is used for further development. Or, the small part and surrounding material are taken out.

This technique is utilized for replicating animals such as Dolly the sheep and potentially for cloning humans in the future. Using SCNT in making exact copies of animals has been hard and has not been very successful. Many babies are dying before or just after birth, which makes the process of giving birth and taking care of newborns not very good. The cloned baby animals have problems with growing and bonding with their parents in non-human species too. For these reasons, and because of moral and ethical concerns, more than 30 countries have banned the cloning of humans. Most scientists think that it won't be possible to use the current cloning method to make a human clone that can grow into a baby in the near future. It might still be possible, but we need to make some changes to fix the problems during the early stages of developing human embryos using SCNT.

There is also a possibility of treating illnesses caused by changes in mitochondrial DNA. New studies found that putting the nucleus of a sick body cell into a healthy egg can stop a disease from being passed down. This treatment doesn't use cloning but could create a baby with genes from three parents. A sperm from a father, an egg from one mother, and an empty egg from another mother. In 2018, scientists successfully cloned primates using a method called somatic cell nuclear transfer. This is the same method that was used to clone Dolly the sheep. Breeding animals from different species using nuclear transfer. Interspecies nuclear transfer (iSCNT) is a way to help save endangered animals or bring back animals that have already died out. The process entails moving the cell nucleus from one species into the egg of a different species. The method is similar to SCNT cloning, which is usually done with domestic animals and rodents, or when there are enough eggs and animals available to use as surrogates. Cloning endangered or extinct species requires a unique method of cloning. Interspecies nuclear transfer is when the nucleus of one animal is transferred into an egg from a different but similar species. In 2000, Robert Lanza made a baby gaur by combining a gaur

with a domestic cow. When a cell nucleus is transferred between species, it demonstrates that the process of reprogramming the nucleus is consistent across all forms of life. For instance, Gupta and his team studied making transgenic cloned embryos by using cells from cows, mice, and chickens and putting them into empty pig eggs. In addition, a special type of cells called NCSU23 medium, originally made for growing pig embryos in a lab, was also able to support the growth of cattle, mice, and chicken embryos in a lab until they reached a stage called the blastocyst. In addition, sheep egg cells can be used to change and reprogram human body cells back to the early stage of development.

CONCLUSION

Stem cells are special cells that can change into different types of cells in the body when needed. Cells from a developing baby, like fibroblasts, can change into any other type of cell in the body. Some scientists successfully made copies of fetal cells to create new animals with specific genes. They made a sheep called Dolly and a bull calf called Gene, along with other animals. Organ transplants, like liver, kidney, or heart, are a very advanced surgery that replaces a bad organ with a healthy one. Using the knock-out approach, researchers may be able to avoid the body's quick rejection of foreign organs. Afterwards, the next issue is when the body takes too long to reject the foreign organ which involves certain cells of the host. Trans-genesis can help stop parasites from causing diseases in people. Many tries have been made in this area recently, but not many have been successful. Schistosomiasis is a sickness caused by a parasite called Schistosoma. It makes people have symptoms like fever, diarrhea, chills, and tummy pain. The parasite gives people sickness when it enters their bodies through their skin while they are in water. Female Anopheles mosquitoes spread a dangerous disease called malaria by carrying Plasmodium parasites. Scientists have found specific genes that are important for the parasite to spread. Bollworm is the name for moth babies that hurt lots of different plants. Scientists created bollworms with a special gene that makes them die. Mediterranean fruit flies ruin fruits and coffee crops all over the world. People are trying to use genetically modified medflies instead of wild ones to help protect crops that people eat.

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CHAPTER 9

DNA BARCODING: EXPLORING THE MOLECULAR STEPS OF DNA

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ABSTRACT:

DNA barcoding techniques were made using knowledge from studying DNA in tiny living things, by using a specific gene. In 2003, new methods for figuring out what species something is by looking at its DNA were suggested. This could also help label unknown DNA sequences as belonging to larger groups of organisms, like orders and phyla. DNA barcoding means figuring out what species an animal or plant is by looking at a small bit of its DNA. Using DNA barcoding, we can compare a sequence of DNA to a database to figure out what kind of organism it belongs to. These “Barcodes” are used to classify unknown species, parts of an organism, or just to create a list of as many different kinds of organisms as possible. They are also used to compare with traditional ways of organizing species in order to define the boundaries of different species. Scientists use specific gene areas to figure out the different groups of living things using a method called DNA barcoding. For example, scientists use the 16S rRNA gene to identify bacteria, the COI gene in mitochondrial DNA to identify animals, the ITS rRNA for fungi, and RuBisCO for plants. When scientists use the DNA barcoding method to group organisms in a sample that has DNA from many organisms, they call it 'DNA Metabarcoding'. For example, using DNA to identify organisms in water samples. The genes were picked as barcodes because they don't change much within a species but do change a lot between different species. This is called the "Barcoding Gap". In this unit, you will learn about DNA barcoding, how DNA sequencing works, and different methods used for DNA sequencing.

KEYWORDS:

DNA barcoding, Gene, Organism, Plant, Species.

INTRODUCTION

By examining a tiny portion of an organism's DNA, DNA barcoding can be utilized to differentiate between species. DNA barcoding is like using a barcode at the store. The process involves comparing the DNA of different species to a reference library to distinguish them. - It can discover unfamiliar species, record various organisms, and make comparisons with conventional methods of species identification. Scientists use different gene regions to identify different groups of organisms using barcoding. The most used barcode for animals and some protists is a part of the COI gene, which is in their mitochondrial DNA. Different genes can be used for DNA barcoding, like ITS rRNA for fungi and RuBisCO for plants. Microorganisms can be identified using various gene regions. The 16S rRNA gene helps identify bacteria, while the 18S rRNA gene is used to find single-celled organisms like fungi and protozoa. These gene regions are picked because they have less differences within the same species than between different species. This is called the "Barcoding Gap". DNA barcoding has a lot of uses [1], [2]. It can help identify plant leaves even if there are no

flowers or fruits. It can also identify pollen on pollinating animals, insect larvae, and help figure out the diet of an animal by looking at its stomach content, saliva, or feces.

When DNA barcoding is used to identify organisms from a sample with DNA from more than one organism, it's called DNA metabarcoding. Using DNA to check the types of diatoms in rivers and streams to see if the water is clean or not. DNA barcoding techniques were created by studying DNA in small organisms to figure out what species they are. In 2003, a standardized way of using DNA barcoding was suggested by Paul D. N to identify different species and put them into groups like orders and phyla. Hebert and others. Hebert and his team at the University of Guelph in Canada showed how useful the COI gene, originally used by Folmer and others, can be. In 1994, scientists used special DNA tools to study different species of invertebrates. They looked at a specific part of the COI gene to see how it varies between different types of animals. The COI has benefits because it is easy to find the sequence and it has both similarities and differences among different species [3], [4]. In a study by Hebert and others, the profiles were referred to as "barcodes". planned to create a database of conflicts of interest that could be used as the foundation for a worldwide system to identify people.

Barcoding can be done by using tissue from a specific animal or plant, a group of different organisms, or DNA found in the environment. Water and soil are naturally occurring substances that are essential for life. The ways to take, keep, or study samples are different depending on the type of sample. To put a barcode on a tissue sample from the specimen, a small piece of skin, scale, leg, or antenna should work. To keep things clean, it's important to clean tools before using them again. It is suggested to take two samples from one organism, one to save for later, and one to use for the barcoding process. Keeping samples in good condition is very important to prevent DNA from breaking down. A bulk sample is a sample of the environment that has a lot of different organisms from the same group. Bulk samples have a lot of good DNA while other environmental samples do not. Some examples of bulk samples are bugs and insects collected from water using a net or a trap. Water samples containing small living things like single-celled organisms may also be called bulk samples when they are sorted by size. The same methods used to collect traditional samples can also be used to collect these samples for identifying shapes and structures [5], [6]. The eDNA method is a way to find out what kinds of animals are in a certain area by collecting and testing their DNA from things like water or soil. It doesn't harm the animals. Analyzing water or soil using barcoding or metabarcoding.

This method works because all living things leave behind DNA in the environment, and we can find this DNA even for organisms that are not very common. When collecting samples from the field, it's important to use materials and tools that don't have any DNA on them to prevent contamination, especially if the target organism's DNA is expected to be in small amounts. On the other hand, an eDNA sample always has the DNA of living microorganisms, which are usually found in large amounts. Microorganism samples taken from the natural environment are also known as eDNA samples. Contamination is not as much of a problem in this case because there are a lot of the organisms we are looking for. The eDNA method can be used to test many different types of samples, such as water, soil, animal poop, stomach contents, or blood. DNA barcoding needs to get the DNA out of the sample, like from leeches. There are many ways to take DNA out of something, and the best way to do it depends on how much it costs, how long it takes, what kind of sample you have, and how much DNA you need. When we use PCR to make more DNA from organisms or from eDNA, there can be molecules in the sample that stop the reaction from working well. It's really important to get rid of these molecules so we can have good quality DNA to study

later. We need to make more copies of the DNA we took out as a necessary part of DNA barcoding. Usually, only a small part of the DNA is studied to get the DNA barcode, which is usually 400-800 base pairs long. The eDNA material is usually amplified to make smaller pieces (<200 base pairs), because eDNA is more likely to be broken into smaller pieces than DNA from other sources. However, some research says that the size of the DNA piece doesn't affect how well it can be detected. Once the DNA is amplified, the next step is to sequence it using DNA sequencing methods. There are many different sequencing platforms to choose from, and the technology is improving quickly.

DISCUSSION

It's no accident that DNA barcoding has grown alongside genomics research. The process of DNA barcoding is utilized for rapid species identification through analysis of their genetic material. Genomics compares the structure and expression of entire genomes. Both focus on collecting a lot of genetic data to answer questions that traditional methods couldn't. DNA barcodes are short sequences of DNA that are the same for all species. They are usually 400-800 base pairs long and can be easily made and studied for every species on Earth. An online library of barcodes will help match the DNA barcode of a sample from the forest, garden, or market. Just like genomics helps us find new genes and compare how genes work, DNA barcoding helps us identify known species and find new ones faster. DNA barcoding tries to identify all different species by looking at just one or a few gene regions. On the other hand, genomics looks at the function and interactions of all genes in one or a few species, like humans. A recent report in PNAS gets us closer to using DNA barcoding in plants [7], [8]. Choosing the right place to put a plant barcode has been more complicated than we thought, and a lot of people have argued about it. Although there is no agreement on a single plant barcode, scientists are thinking about using a genetic identifier for many research and projects related to plants.

The study shows that we can use plant DNA codes to figure out which species are in danger in important areas for biodiversity. We could also use this information to keep track of the trade of rare orchids. Researchers are currently counting all the different types of plants in forests using their DNA. They are doing this in places with mild weather like Plummers Island in Maryland and a park in New York, and also in tropical places like Forest Dynamics Plot in Panama and soon in La Selva Biological Station in Costa Rica. This will help scientists identify plant pieces in nature studies and compare genetic differences between different forests. If the barcode label includes the right gene, it can help scientists make family trees for all the plants in a forest. This can make it easier for them to study how the plants in the forest are related and how they have changed over time. The Forest Dynamics Plot is in a group of 20 places in warm countries that have a lot of trees. Together, they have almost 3.5 million trees, which is about 12% of all the types of trees we know about [9], [10]. A full count of the DNA barcodes of all the trees at these locations is now being organized. The germplasm bank created from this global DNA barcoding project will create new chances for studying DNA, including community evolution and ecological genetics.

The markers used for identifying DNA are known as barcodes. It's really important to pick the right DNA parts so we can tell species apart using DNA barcodes. A reliable DNA barcode will exhibit diversity within individuals of the same species, while displaying marked distinctions between various species. It must also contain consistent sections of DNA to be applicable across various organisms. The aim is to create primers that can find and tell apart almost all the different types of organisms in the group being studied. The barcode sequence should be short so that it can be used with current methods for getting and studying DNA. It would be best if one gene sequence could be used for all different types of living

things, like viruses, plants, and animals. So far, no specific gene area has been discovered, so various codes are used for different groups of living things or for different study questions. The most common barcode for animals is a part of their mitochondrial DNA called COI. Other parts of mitochondrial DNA, like Cytb, 12S, and 16S, are also used for barcoding. Mitochondrial genes are better than nuclear genes because they don't have introns and are easier to inherit. Also, each cell has many mitochondria and each one has several DNA molecules. Mitochondria can provide a lot of DNA even if there is only a small amount of tissue available.

In plants, mitochondrial genes don't change much, so they can't be used for identifying plants. But some genes in the chloroplast, especially the maturase K gene (matK), might work for this. Complex genetic markers like ribosomal internal transcribed spacers (ITS DNA) and other genes are used to identify different plant species. It works best when using two or more chloroplast barcodes. Bacteria can use a small part of their genetic material called the 16S gene to identify different groups, because it doesn't change much over time. Some studies suggest that other parts of bacterial DNA, like COI, cpn60, and rpoB, could also be used. Identifying fungi with barcodes is hard, and sometimes we need to use more than one set of starting materials. The COI marker works well for some fungi, but not for all of them. That's why we use other markers like ITS rDNA and 28S LSU rRNA[11], [12]. In protists, different barcodes have been suggested, like parts of the 28S rDNA, 18S rRNA, ITS rDNA and COI. Also, certain barcodes can be used for small organisms that make their food from sunlight, such as the rbcL gene and 23S rRNA gene in the chloroplast.

For a gene to work as a DNA barcode, it needs to have three things It must have a lot of genetic differences between different species, It should have similar areas around it so that it can be used for genetic tests on a wide range of species, and It should be short so that it is easy to work with in the lab. A small piece of DNA that is 600 base pairs long in the gene for a part of the mitochondria has been chosen as a standard way to identify different species of animals. You can find more information at www. siedu Plants can't use CO1 as a barcode, so botanists are trying to find a better marker. Some gene regions have been suggested as barcodes for plants, but the taxonomic community has not agreed on any of them. There is no agreement because the plastid marker for plants is limited compared to CO1, and there is no clear method for choosing a gene to use as a barcode for plants. When choosing a plant DNA barcode, it is important to consider a few things: making sure it can be amplified through PCR, covering a wide range of plant types, being able to tell different species apart, and being useful for analysis and computer applications.

Compare different parts of plants DNA that have been suggested as barcodes with their top choice, the plastid gene matK. Their paper has all the important things: they studied many different kinds of flowering plants, used a primer set that works for more plants, tested their method for identifying and finding new species, and used barcodes to help with environmental problems. This article is good, but like many other articles that suggest plant barcodes, the authors don't give specific measurements and rules that are needed to compare their favored barcode with others. The most important thing when choosing a DNA barcode is that it can work for many different plants. A hard choice exists between making sure PCR works for all samples and having a lot of differences in the genetic sequences. This balance between different factors is a bigger issue in coding areas, but not as much in noncoding areas. This is because standard starting points are usually found in the genes that surround the variable spaces in between them. The scientists are not sure about how to choose the right levels for making plant DNA barcodes and how to make the PCR conditions simple for all plants. DNA barcoding needs to be easy for many people to use. The way it is done should be

simple and accessible for everyone. The more information we have in the DNA barcode library, the better DNA barcoding will work. If we have a complete database, DNA barcoding will be very powerful. These things need specific PCR conditions and primers to make a good barcode for different plants and animals.

They say they tested their barcode locations on the most types of organisms ever used in a study. While there are many different types of plants in the study, almost all of them belong to one family called Orchidaceae. The other examples come from 23 different families in 18 groups, which is less than half of the families and groups in earlier tests. Also, they said they used many different primer pairs instead of just one to make sure they could amplify matK in all the samples. MatK has not been shown to be a good barcode for plants because it is not widely used and easy to use. A measure of how well a barcode works for different kinds of plants, like flowers, trees, ferns, and mosses, is called PCR universality. We only tried using matK on flowering plants and we said it's okay if it doesn't work for all types of plants. In the ecosystems today, most of the plants are angiosperms. Some people think we should pick markers that work best for these popular plants. Since the reason for a DNA barcode is to help identify unknown samples, even small pieces of plant tissue, the chosen DNA markers should be able to work well for all types of green land plants. In simple terms, any pattern in DNA that is different between two species can be used as a barcode to identify them. In addition, DNA barcodes do not need to show similarities in mutations like other markers used for studying evolutionary relationships. In simple terms, small differences between species can still help to tell them apart, even if they don't show their evolutionary relationships. In animals, the differences in CO1 are bigger between species compared to plants [13], [14]. Interestingly, the gene region called trnH–psbA had a high difference from matK, which had almost 50% less difference between different species. So far, there is no measure to compare barcode options. We can use a simple statistic to compare different DNA barcode markers by multiplying PCR universality and sequence divergence levels. The percentage of species that can be tested and the percentage of species that can be told apart by a specific gene can be multiplied together to make one number for comparison.

The basic comparison statistic suggested is only important when we also think about other things, like how hard it is to find the PCR amplicon and how many different primers and reaction conditions are used when sequencing each possible barcode spot. We can figure out if a certain gene is good for testing lots of DNA by comparing it across different species under the same testing conditions. This statistic helps us understand how well a DNA barcode can identify different species in a bioinformatics context. It looks at the balance between how well the barcode can be amplified and how much it changes across different groups of species. In the end, the way the database is set up and the strategies used to search for sequences can really influence how well barcode markers are able to identify and discover species. So far, we haven't figured out the best way to search the barcode database, especially when it comes to using a multilocus DNA barcode. The way GenBank and BOLD databases work is not the same. Many different ways to line up sequences are available for DNA barcodes, and they can be tested for use based on: how confident we can be in assigning a species, using only part of a sequence when searching a database, and how variations in sequence length and mutations can affect search results. DNA barcoding has a lot of potential for helping scientists learn more about animals and plants and how they have changed over time. It's important to pick the right genetic markers for this to work well [15], [16]. The problems mentioned here, if we think about them carefully and put them into action, will help us choose a plant DNA barcode using a fair comparison and measurements.

Reference libraries are used to figure out what kind of organism a DNA sequence comes from. It's also called annotation. The sequences are usually from barcoding or metabarcoding. These databases have the DNA barcodes for known species. Many libraries don't have information on all types of animals, and new information is always being added. For big and small organisms like algae, these libraries need a lot of information like when and where it was found, who found it, and a picture. It also needs to be identified by an expert and have its genetic information submitted in a specific way. However, these standards are only met by a few species. The process also needs to store samples in museums, plant collections, and other places that work together. Having a lot of information and making sure it's good are both important for identifying things accurately. In the world of tiny organisms, we don't have DNA information for most species names, and many DNA sequences don't fit into the usual naming system. Depending on the type of organism and genetic marker, there are different databases we can use for reference. There are smaller databases for specific countries such as. FinBOL and iBOL are big groups of scientists working on the Barcode of Life Project.

It is a place where people can store and use information about specimens and genetic codes for studying barcodes. It helps with organizing, checking and working with barcode data. The database has information about animals using a genetic marker called COI. At BOLD, they use sequences from matK and rbcL to identify plants. The UNITE database was created in 2003. It helps identify different types of fungi and other organisms using a specific genetic marker. This database is built on the idea of species guesses: you pick the percentage you want to use, and the sequences are arranged and compared to sequences from specimen identified by experts. It had information from two places: the Thonon culture collection (TCC) at the hydrobiological station of the French National Institute for Agricultural Research (INRA), and from the NCBI nucleotide database. Diatbarcode gives information about two genetic markers, rbcL and 18S. The database also includes more information about species like what they look like size, how they move, how they live together and how they are affected by pollution. To get data that is organized, clear, and easy to understand, we need to use bioinformatics to analyze the raw sequencing data. The FASTQ file contains both the DNA sequences present in the sample and the corresponding quality scores for each nucleotide in the sequences. The PHRED scores show how likely it is that the nucleotide has been scored correctly.

CONCLUSION

DNA barcoding helps to identify unknown organisms at any stage of their life using molecules. This technology is applicable to various organisms at any stage of their life. Using this method, scientists can analyze tissue from the initial stages of development in seeds or embryos up to the mature adult organism. This technology helps to collect fewer organisms from the field and makes it faster to identify them. Barcoding can help make special primers and tests for different types of species found in the environment. We will keep working to solve the problems with barcoding as we analyze more samples at RDLES. In the past year, RLDES has proven that it can analyze barcoding samples from many different types of animals. This new method will help many Reclamation projects by quickly and affordably identifying different species.

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CHAPTER 10

APPLICATION OF PHEROMONES IN ANIMAL BREEDING AND CULTURE

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ABSTRACT:

Pheromones are substances emitted by animals that prompt similar species to exhibit specific behaviors. Animals and insects use chemicals to talk to each other. Pheromones may consist of carbonic acid, steroids, aldehydes, ketones, alcohols, and other chemicals. Insects use pheromones as chemical signals to find food, attract a mate, or warn others about predators. By using specific scents, traps can be used to track and monitor pests in farms and homes. This could reduce harm to crops, plants, and houses and buildings. This can also reduce the number of stinging bugs in the neighborhoods close by. Pheromones can also help to locate where the pests are starting to live. For example, bugs like Asian gypsy moths and Japanese beetles can harm plants, but we can use community traps to control them. The word "pheromone" was made up by Peter Karlson and Martin Lüscher in 1959. " Pheromones are sometimes called ecto-hormones. They were studied by different scientists before, such as Jean-Henri Fabre and Joseph A. Lintner, Adolf Butenandt, and ethologist Karl von Frisch gave different names to these, such as "Alarm Substances". These chemical messengers travel around the body and can affect how our nervous system, immune system, and behavior work. In this section, you will learn about how pheromones are used to control pests, insects, and rodents. You will also learn about how pheromones are used in breeding animals and in conserving and managing indigenous cows, buffaloes, tigers, and elephants.

KEYWORDS:

Animals, Chemical, Pheromones, Sex Hormones.

INTRODUCTION

Insect pests are more effectively controlled using pheromones compared to rodents. Rodents' behavior is more complicated, which makes it difficult to study how pheromones affect them and how they can be used to control them. However, pheromones are important for rodents. In mice and rats, pheromones can be put into two main groups: Signal pheromones are substances that cause animals to react quickly. Primers are things that cause changes in the body after a while by affecting the brain and hormone system. Pheromones that signal the release of rodents are showing more potential in controlling them. These pheromones make animals attracted to each other for mating and can also make them act aggressively. The smell of female rodent pee or discharge helps attract males to traps or stations with poison. Additionally, a smell made by male rodents that attracts female rodents could be very helpful in controlling rodent populations [1]. For example, mice have glands that make a special substance that attracts female mice who want to mate.

Pheromones can be put into different groups based on what they do. Aggregation pheromones are chemicals that bring together a lot of male and female animals of the same kind.

Aggregation pheromones are used by an animal to work together with others, like when they are trying to move into a new home. These scents also do other jobs like picking a mate, overcoming the host's resistance by attacking in large numbers, and protecting against predators. Aggregation pheromones are a way to control pests that are good for the environment. Alarm pheromones can make animals run away or fight each other. Alarm pheromones are also used to tell other animals of the same kind about coming danger. For example, aphids use a smell to tell other aphids that a ladybug attacked them. When certain plants get eaten by animals, they make a substance called tannin. The tannin makes nearby plants taste bad to the animals, so they won't eat them. Releaser pheromones are chemicals that make someone's behavior change. Releaser pheromone makes the body react fast, but it doesn't last long. For example, some beings use strong-smelling chemicals to attract partners from far away. Signal pheromones cause temporary changes in the body, similar to how neurotransmitters work. First, there are different types of pheromones. The "primer" pheromones make changes in how something grows and develops, while other pheromones change how something behaves [2], [3].

Pheromones let other animals know where an animal's territory is. Some animals like cats and dogs have pheromones in their urine that they use to mark their territory. Trail phjsones are used by insects like ants and honeybees to communicate with each other. For example, ants leave a scent on their paths so they can find their way back. This path brings in other ants and shows them where to find food. Sex pheromones are chemical signals that make animals attracted to each other for mating. When female animals release special scents, it means they are ready to have babies. Male animals can also release chemicals that tell other animals about their kind and genes. Female animals release pheromones to attract male animals of the same kind. Male animals release pheromones to attract female animals of the same kind. Parasitoids use host-marking pheromones to avoid laying their eggs on hosts. Hyper parasitoids can use host-marking pheromones to find their hosts [4], [5].

Pheromones are chemicals released by an animal that affect the behavior of others in the same species. They are the chemicals that insects and other animals use to talk to each other. Pheromones are mainly used to help cows and pigs breed. They are sold in stores as products that are similar in structure. However, on some farms, nose rings are used with chemicals that contain pheromones. This is pee and booger from cows when they are ready to have babies. Pheromones are important in making animals behave a certain way or affecting how they reproduce by changing the levels of hormones in their bodies. Because of this, pheromones are also called ectohormones, which are chemicals that are sent outside the body to send messages. They make the opposite poles more attractive and promote fighting between males. They also make puberty happen faster, shorten the time when females can't get pregnant, change the cycle when females can get pregnant, and make both males and females want to mate [6], [7]. Also, pheromones have been seen to have a positive effect on the reproductive ability of cows and other farm animals when breeding bulls are around. Using a nasal spray with pheromones increased the amount of sperm and made them healthier and able to move better in farm animals. So, after talking about it, we can say that pheromones are important for animals to breed naturally, and also for technologies like artificial insemination and MOET or ETT.

DISCUSSION

Pheromones are chemicals that animals use to talk to each other. The term "pheromone" comes from two Greek words: "Pheran" which means to transfer, and "Hormon" which means to excite. The term was first made up to describe when one animal releases substances that cause a reaction in another animal of the same kind. This could be a specific behavior or

a process of growth and change. These are chemical molecules that are released in humans, insects, and animals to make them react in a certain way or to cause specific changes in behavior or hormones when they are around others of the same or opposite sex in their species. " Biostimulation is when males make females more likely to go into heat and release eggs through touching smells, or other signals. The author said that having male animals around can make puberty start earlier in some mammals. In cows, the time between giving birth and being able to give birth again can be shorter if they are around vasectomized bulls. Since then, many studies have found that male cows being around after giving birth makes female cows have regular cycles. The way pheromones affect cow reproduction is not as well understood as it is in other animals like sheep, goats, and pigs. The information learned about how bio stimulation works in farm animals, and what affects it, helps people use it to help breed animals better. Using pheromones can help animals have babies sooner and not have as much time in between having babies [8], [9]. They wanted to understand where these signals come from, what kinds there are, and how they affect the animals' bodies. This article aims to explain the importance of these signals in farm animals. Pheromones are chemicals that come out of the body in fluid and waste. Usually, pee, poop, fluids from the cervix and vagina, and other bodily secretions are the main sources of pheromones in animals with backbones. Urine is a type of liquid that mammals use to communicate with each other.

It has smells that can make animals feel interested in mating. This is true for many different kinds of animals, including farm animals. Urine has many different chemicals in it that can make it difficult to find pheromones. In 1986, it was thought to be an important signal for when animals are ready to mate. Evidence shows that different animals like water buffalo, pigs, sheep, and goats have their own special scents called pheromones. Buffalo and mare poop. In 2006, scientists studied the spit from pigs and cows, the wax from sheep, and glands in their mouths. Artificial insemination is when we take sperm from a really good male and put it inside a really good female to help them have babies. There are three ways to do artificial insemination: Vaginal method, Recto vaginal method, The speculum method. The Recto vaginal method and the speculum method are best for beef cattle because of their reproductive tract size and structure. Below, we will talk about two methods in detail. This is a popular and safe way to check cows in the dairy industry. It's practical and well-liked by farmers. This method involves touching and moving the cervix through the rectum [10], [11]. This method involves putting a speculum into the vagina and pushing it up to the back of the cervix.

Advantages of Artificial Insemination It helps to use the best bulls more efficiently, it allows for better selection of genes, it's less expensive in the long run, It benefits both farmers and animals, It lowers the risk of spreading diseases. Disadvantages of Artificial Insemination. It requires skilled workers to use the technology, Farmers need to choose the best male animal for the process, It can reduce genetic diversity over time. The initial Embryo Transfer Technology (ETT) project in India was initiated by NDDB (National Dairy Development Board) in 1987 at Sabarmati Ashram Gaushala (SAG), Bidaj. The government's Department of Biotechnology funded the project for 5 years. from April 1987 to March 1992. from April 1987 to March 1992. NDDB started a project and set up one main lab and four regional labs in different states for embryo transfer. NDDB helped set up 14 State ET centers in the country.

Embryo Transfer (ET) or Multiple Ovulation and Embryo Transfer (MOET) helps female dairy animals have more babies. It is used to increase the number of offspring from the best genetic cows. Normally, a good female dairy animal has one baby calf each year. But with 'MOET'/'ET' technology, dairy workers can help a cow or buffalo have 10-20 calves in a

year. A special cow/buffalo is given hormones to help it produce more eggs. The FSH-like hormone makes the genetically superior female produce many eggs instead of just one. The female is given medicine to help her release eggs, and then she is fertilized with sperm from a male. This is done two or three times, 12 hours apart, when she is ready to have babies. After 7 days, the inside of her body is rinsed with liquid to find the baby animals that are starting to grow. Embryos are gathered with a special liquid in a certain filter. After that, we look at the quality of the growing embryos under a microscope. High-quality embryos are either saved or frozen to use later, or they are put into a surrogate mother about seven days after she goes into heat. So, by using 'ET' technology, many better calves can be produced each year. SAG has achieved significant accomplishments in this area, producing 14,388 embryos and 755 calves, the highest numbers in the country. Out of these, 1026 embryos are from local cows, and 122 baby cows have been born [12], [13]. In addition, about 3000 buffalo embryos have been created. In 1991, the first Indian buffalo calf was born from a frozen embryo as part of a project.

They cover 14% of the country's farmland and make up almost 3% of the country's total income. They also contribute about 6% of the value of all agricultural products. Oilseed crops in India don't grow as well as they do in other parts of the world. One reason for this is that they are easily damaged by insects. The usual way to control these bug pests is by using man-made bug-killing chemicals at different times from when the plants are put in the ground until they are picked. The careless and unwise use of strong insect-killing chemicals caused insects to become resistant to the chemicals. This led to more pests, and also harmed animals and insects that the chemicals were not meant to affect. In situations like this, we should use less insect-killing sprays and instead keep an eye on the insect population [14], [15]. Then we can use the sprays at the right time when the insects are most vulnerable.

New studies have shown that pheromones could help keep an eye on pests and control them better. Pheromones are chemicals that one animal releases to make another animal of the same kind do something specific. Pheromones are divided into different groups based on what they are used for. The most common types used to manage pests are. Sex pheromones are chemicals that animals use to find a mate and attract them for courtship. One gender's actions make the other gender do certain behaviors, which helps them mate. Female organisms usually produce it to attract male organisms for mating. In some types of bugs, the male bugs make a scent that brings the female bugs to them. Sex pheromones are made up of single molecules or a mix of compounds in a certain ratio. The sex pheromones emitted by Lepidoptera are the most studied and used in IPM. Aggregation pheromones make insects come together in one place to eat or make babies. This could help animals find a mate, stay safe from predators, and make sure they get enough food. These smells are mostly given off by beetles.

The alarm smell makes insects run away quickly when they sense danger. These smells are found in insects that live in groups. They are in bugs like Aphids and Thrips, which can cause a lot of damage to crops. Social insects use trail pheromones to mark the paths to food and their nests. Pheromones that mark a host help parasitoid to avoid fighting with others of the same kind when they lay eggs. Sex pheromones and group pheromones are used to watch and control bugs in oilseed plants. Setting up traps with pheromone lures can help us find pests early, keep track of how many pests there are, figure out where they are, examine them for quarantine rules, estimate how many pests there are, and decide when to take action based on the number of pests. 10-12 traps per hectare with the sex pheromone of tobacco caterpillar have been suggested for checking the pest in soybean, castor, groundnut, and sunflower fields. A special trap with a smell that attracts bugs has been suggested to use to keep track of

a bug called the gram pod borer in soybean, groundnut, and sunflower fields. It's recommended to put 10-12 traps in every hectare of land. Pheromones work well to control pests by catching them in large numbers or messing up their ability to mate. We can use many traps with special scents in the fields to catch male moths when they first come out. This can help decrease the number of males that can mate.

A trap with a special smell can catch a lot of leafminer moths and help reduce the number of them in groundnut fields. Using specific scents to attract white grub beetles to a certain plant has been found to be helpful in controlling the pest in groundnut and other crops. The chemical anisole, found in the stomach glands of female Holotrichiaconsanguinea bugs, can attract both males and females from 15 meters away. Using this pheromone has made it easier and safer to control beetles. Insecticide spraying and "Pheromone Dispensers" are only necessary for one tree within a 15-meter range, resulting in cost savings. To do this, we can choose one tree out of a group of trees that are within 15 meters of each other. Then we can spray that tree with imidacloprid 17.8 SL or quinalphos 25 EC at a concentration of 1.5 ml per liter during the day. "Pheromone dispensers" are small devices that release a scent. They will be put on the tree (3-4 devices per tree) at night for three nights after the beetles come out. This method was good at bringing in and getting rid of the beetles and cutting down on the white grub problem.

Different pheromones have different purposes and work in different ways. Some are used to attract a group of insects, while others are used to warn them of danger. There are also pheromones that help insects find their way or communicate information, as well as ones that are related to mating. These make animals act differently for a short time. They can attract or repel other animals. These pheromones make animals behave in a different way. For instance, some living things use strong-smelling substances to attract partners from far away, even up to two miles. Generally, this kind of smell makes a quick reaction happen, but then it goes away fast. The male pig makes the female pig still by using special chemicals in its spit. They are made in the testes and come out in a boar's spit. First pheromones: They take longer to start and last longer. For example, mother rabbits release a smell from their bodies that makes their babies want to nurse right away. They cause lasting changes in behavior or development by activating a certain part of the brain. Bull urine can make young female cows reach puberty sooner. More of the cows that were treated with bull urine every week reached puberty compared to those that were treated with water. Priming pheromones can change how our bodies work by either slowing down or speeding up different systems like hormones and reproduction.

In pet animals, the smell from the male animal can affect when the female starts to have babies, when she is not able to have babies for a while, and how long it takes for her to have babies again after already having some. Each of these effects makes female animals come out of a period of not being able to reproduce by using a chemical called pheromone. Being around a grown-up male pig makes young female pigs reach puberty sooner and have their first heat at the same time. Giving post-pregnancy sows a fake signaling smell called 'Boar Mate' makes them come into heat sooner. This was shown in a study by Hillyer in 1976. Signal pheromones make things happen quickly, like releasing chemicals that trigger a reaction in the body. For example, the Gonadotropin-releasing hormone (GnRH) molecule acts like a messenger in rats to make them show lordosis behavior. A special scent makes animals act right away and works through the nervous system. During the parade, a young bull smells special chemicals from the female cows and makes a funny face. Simple rephrasing: Information about how bulls act around cows can be the most trustworthy way to

tell if pheromones are present. This can help scientists understand the chemicals involved in communication. This is important for animals to recognize their parents and to choose a mate.

Grouping pheromones help protect against enemies, choose a mate, and overcome resistance from the host by attacking together in a large group. A bunch of people in one place is called an aggregation, whether they are all one gender or both genders. Men make substances that attract other animals for mating. These substances are called aggregation pheromones because they bring both male and female animals to the same place and make more animals gather around the source of the substance. Grouping together chemicals (aggregation pheromones) is one of the most specific ways to control pests in nature. They are safe and work well even in small amounts. Sex pheromones tell male animals when a female is ready to mate. Male animals can also release pheromones that tell other animals about their type and genes. Female insects make most of the sex pheromones, but some are also made by male insects. Controlling the release of pheromones: The inside of a mammal's nose has two different parts, the main smell area and the vomeronasal organ. The MOE can analyze different smells and chemicals in the environment, but it doesn't have a specific purpose. The VNO can detect special chemical signals that tell us about an animal's gender, reproductive or dominance status. The VNO has receptors that get turned on by certain chemicals or pheromones. These special nerve cells likely trigger a series of hormone responses that happen without the person realizing it.

CONCLUSION

Animals use scent to talk to each other and do important things like have babies. Cattle pheromones can help improve how cows reproduce and are taken care of. To make sure dairy cows have babies at the right times, produce lots of milk, and have lots of babies, farmers use artificial insemination to help them reproduce. Accurately finding the best time for AI can be done by detecting when an animal is in heat using pheromones. The bull plays a very important role in helping cows to start having babies again after giving birth. So, we need to study more about how these parts work with the reproductive system of cows after they give birth. The pee smell can make a hormone in the brain work better. We should study more about how this works and how it could help with making babies in the future.

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CHAPTER 11

STEM CELLS: EXPLORING THE ROLE OF CELLS IN TISSUE REGENERATION

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ABSTRACT:

The words "stem cell" were made up by Theodor Boveri and Valentin Haecker in the late 1800s. Important research on blood stem cells was done at the start of the 1900s by ArturPappenheim, Alexander Maximow, and Ernst Neumann. In the University of Toronto during the early 1960s, Ernest McCulloch and James till made a groundbreaking discovery about the essential qualities of stem cells. They found a cell that makes blood, called the Hematopoietic Stem Cell (HSC), by doing important research on mice. McCulloch and till did some tests where they put bone marrow cells into mice that had been exposed to radiation. Stem cells are special cells that can change into different types of cells in the body and keep making more of themselves. They are the first kind of cell in a cell family. They are found in both young and older organisms, but they work a little differently in each. Stem cells are different from cells that can't divide forever, and cells that are already committed to becoming a specific type of cell. Grown-up stem cells are only in certain parts of the body, like in the bone marrow or reproductive organs. They are here to replace lost cells quickly. They can change into a few or just one type of cell. Mammals have different types of stem cells. Some help make blood and immune cells, some help keep the skin healthy, and others help maintain bones, cartilage, muscles, and fat. Adult stem cells are a small group of cells that are outnumbered by the cells they turn into. In real life, stem cells are recognized by their ability to make new tissue. For instance, the main test for bone marrow or Hematopoietic Stem Cells (HSCs) is the ability to move the cells to another person and help them if they don't have HSCs.

KEYWORDS:

Blood Cells, Bone Marrow, Gene Knock, Knock Technology, Stem Cells.

INTRODUCTION

Stem cells are special cells that can divide and change into different types of cells in the body as needed. As shown in the picture below, they can change to make different kinds of cells. They are cells that are not specialized for a specific job. "The second text should be rewritten using simpler words. " They can make copies of themselves by splitting into two different cells. Stem cells can change into different types of cells as the body needs them. They do this in response to signals they receive. There are different types of stem cells based on how well they can turn into different kinds of cells. Totipotent stem cells can turn into any kind of cell. Eggs and sperm are special cells in our body that can turn into any kind of tissue. The second text is requesting simplification. Pluripotent cells are able to become many different types of cells in the body. Cells taken from the early embryo, called blastocyst, are known as pluripotent. Multipotent cells can only turn into cells that are similar to them, from the same tissue they come from [1], [2]. For instance, Grown-up blood stem cells can change into red

or white blood cells or platelets. Initially, mesenchymal stem cells (MSCs) were described as cells that stick to surfaces and look like fibroblasts. They can change into different types of cells like bone cells, cartilage cells, fat cells, tendon cells, and muscle cells. MSCs can come from the tissue that holds our organs and tissues in place. MSCs can stay usable for a long time without losing their strength.

Because they can make more of themselves, Hematopoietic Stem Cells (HSCs) can change into all different types of blood cells. They can help with blood disorders. This picture shows how HSCs divide. Neural stem cells are in specific areas in the adult mammal brain. Because they can turn into many different types of cells and can keep making new cells, they could be used to treat brain problems with cell therapy. Stem cells in the digestive system are found in the intestines and stomach. But, many people argue about where the stem cells are and how they work. Skin cells are always getting worn out and need to be replaced quickly. The outer layer of the skin has special cells that help it heal and stay healthy. Epidermal stem cells are found in the bottom layer of the skin and they can renew themselves. They play a big role in keeping the skin healthy and helping it heal from wounds. They can also contribute to the growth of abnormal cells that can form tumors [3], [4]. The liver can heal itself and use different ways to do it, depending on the type and severity of the damage. Grown-up liver cells can grow to fill in for damaged tissue and help the liver work better again. These grown-up stem cells can keep making more of themselves without stopping. Therefore, it can be said that an adult's body has stem cells all the time, and the body can use these stem cells when it needs to and when it gets a signal to do so. Adult stem cells stay the same until the body needs them to fix or maintain body tissues. But it is hard to find and separate adult stem cells. They can stay in a state where they aren't dividing or doing anything specific for a long time. Then, the body tells them to fix, grow new tissue or make more of themselves [5], [6].

Under the best conditions, stem cells can fix damaged tissue when they get the right signals. This could help a lot of people by healing wounds and repairing tissue damage after illness or injury. Below, we have listed some ways that stem cells can be used. Stem cells are often used to help the body repair damaged tissues. Up until now, if someone needs a new kidney because their old one is damaged, they had to wait for someone to donate a kidney before they could get a transplant. But if you have stem cells, you can tell them how to change to make a specific part of the body. For example, a doctor uses special cells from just under the skin to make new skin. These new cells can help heal wounds and burns on the skin. In 2013, scientists made blood vessels in mice in a lab using human stem cells. It was found that new blood vessels formed after 2 weeks of putting in the stem cells [7], [8]. Scientists thought this method could be improved to help those with long-term blood vessel and heart diseases. Scientists are working on changing embryonic stem cells into certain brain cells and tissues. They hope to use these cells to treat brain diseases like Parkinson's and Alzheimer's. Scientists are working on making strong heart cells in a lab to help people with heart problems. These new cells that were moved to another place could quickly fix damage to the heart. In the same way, people with type I diabetes could get new pancreatic cells to make insulin because their immune systems have damaged their own insulin-making cells. Scientists use special adult cells to help people with sickle cell anemia, leukemia, and problems with their immune system. Hematopoietic stem cells can make copies of themselves and change into different kinds of blood cells like red blood cells, which carry oxygen and carbon dioxide in the blood, and white blood cells, which fight diseases. Stem cells are used in research to help with healing, cell growth, and testing new drugs on organs made from stem cells, instead of on people. They can also help us understand how cells work and why they sometimes become cancerous.

DISCUSSION

The importance of stem cells for living organisms cannot be overstated, as they serve many vital functions. In a 3- to 5-day-old embryo, called a blastocyst, the inner cells make all parts of the body, like the heart, lungs, skin, and other organs. In certain parts of the body like bone marrow, muscle, and brain, special adult stem cells make new cells to replace ones that are lost because of everyday use, injury, or sickness. Stem cells can help treat diseases like diabetes and heart disease because they can regrow and repair damaged tissue. But there is still a lot of work to do in the lab and the hospital to figure out how to use these cells for treating diseases, which is also called regenerative or reparative medicine. Scientists study stem cells in a lab to understand what makes them special and different from other types of cells. Researchers are using stem cells in the lab to test new drugs and to create models to study how things grow and find out why birth defects happen. Scientists are still studying stem cells to learn how living things grow from just one cell and how new cells can replace old or damaged ones in adults [9], [10]. Studying stem cells is very interesting, but it also comes with a lot of questions and new findings. These cells are usually removed from the hip or pelvic bone.

Specialists remove the stem cells from the bone marrow to donate or save them. The person donating gets a lot of shots that make their bone marrow release stem cells into their blood. Afterward, some blood is removed from the body, and then the stem cells are taken out from the rest of the blood using a machine. Then the clean blood is put back into the body. This is a safe and painless method where stem cells can be easily collected from the umbilical cord after a baby is born. Some people give their cord blood cells to help others, while others save it for later. Knock-in and knock-out technologies are very different. These tools and techniques are used to remove or turn off a part of the DNA. Knock-ins change one part of the DNA in the genome. The knock-in technology focuses on a specific location in a sequence, while knock-out mouse technology creates a completely new DNA sequence. A gene knock-in (KI) is a method of changing the DNA in a gene. It can replace or add new DNA in a specific place in the gene. Usually, this is done in mice because the technology for this process is better and there are many similarities in the genetic code between mice and humans. Knock-in technology is when a gene is put into a specific spot, while traditional transgenic techniques are not as specific. It is the opposite of switching off a gene. Knock-in technology is often used to make models of diseases. It is a way for scientists to study how the regulatory machinery works [11], [12]. The new gene follows the rules set by the old gene it's replacing. This is done by looking at the new appearance of the organism.

The BACs and YACs are used here to move big pieces of DNA. Knock-in technology is not the same as knock-out technology. Knock-out technology tries to delete or insert DNA to stop a specific gene from working, but knock-in technology adds new DNA to a specific spot on the DNA. Gene knock-in technology changes the genetic location of interest by replacing one DNA sequence with another or by adding new sequence information to it. A gene knock-in is like adding a new function to a gene, while a gene knock-out is like losing a function. A gene knock-in can also involve replacing a mutant gene with a normal one, which may result in some loss of function. Gene knock-in started as a small change to the original knock-out method made by Martin Evans, Oliver Smithies, and Mario Capecchi. In the past, knock-in methods used homologous recombination to replace specific genes, but there are also other ways to insert the target gene using a transposon system. The loxP sites are used in gene vectors and removed when Cre-recombinase is expressed [13], [14]. This is an example of genetic engineering. We put the modified embryonic stem cells into a growing blastocyst. It becomes a chimeric mouse with some cells having the blastocyst's genes and others having

the modified stem cells' genes. The babies of the chimeric mouse will have the gene knocked in. Gene knock-in has helped researchers study how specific gene changes affect the characteristics of living things. This was not possible before. Changes in the human p53 gene can happen when exposed to a chemical called Benzo(a)Pyrene (BaP). This changed gene can then be put into mice. The lung cancers seen in the genetically modified mice help prove that BaP can cause cancer. New techniques have been developed to put a gene for green fluorescent protein into pigs using CRISPR/Cas9. This makes it easier to put genes into pigs. CRISPR/Cas9 can quickly make changes to genes in mice, allowing researchers to see the effects in just one generation, which has never been done before. Due to the success of gene knock-in methods so far, we can imagine many ways they could be used in medicine. Scientists have put parts of the human immune system into mice. This lets the mice make antibodies that can be used to treat people. We might be able to change stem cells in people to fix faulty genes in certain body parts. For example, we could fix the Gamma-chain gene in stem cells to help people with a weak immune system. Although gene knock-in technology is useful for creating models of human disease and understanding proteins inside living organisms, there are still many problems with it. Many of these things have the same limits as knock-out technology [13]. When new genes are added to an organism, it can make things more complicated and cause unexpected problems. This can happen when the new genes interact with other genes in the organism. Furthermore, only a small number of places in the genetic code have been well studied, like the ROSA26 place, where they can be used to add genes in a specific way. This makes it difficult to put both reporter and transgenes in the same place. One problem with using gene knock-in to create disease models in humans is that mice and humans have different physiologies. This means that proteins in mice may not fully represent how a gene works in human diseases.

The presence of this trait can be observed in mice with the ÄF508 fibrosis mutation in the CFTR gene. This mutation is found in more than 70% of people with cystic fibrosis. ÄF508 CF mice show the same processing problems as people with the mutation, but they don't have the same lung issues and their lungs look normal. Using different animal models, such as pigs, could help improve these problems. Pigs have lungs that are very similar to human lungs, so scientists are using them to study a certain mutation.In the past, homologous recombination was the main way to disable a gene. This method involves making a DNA structure with the mutation we want. To make a knock-out, a drug resistance marker is usually put in instead of the gene we want to remove. The construct will also have at least 2 kilobytes of similarity to the target sequence. The thing can be given to stem cells by using a small injection or by using electrical pulses. This method uses the cell's natural repair processes to put the new DNA into the existing DNA. This changes the order of the gene, and in many Stem Cells cases, the gene will make a protein that does not work, or may not make a protein at all. However, this process is not very effective because only a small percentage of DNA integrations are caused by homologous recombination [15], [16]. Usually, the drug marker on the genetic material is used to choose cells where the change has happened. Site-specific nucleases are tools that can be used to cut DNA at a specific location. There are currently three methods for doing this. When a cell gets a double strand break, it tries to fix it using a repair method called Non-Homologous End Joining. This method involves joining the two broken ends of the DNA directly. This can be done not perfectly, which can add or remove base pairs, causing frame shift mutations. These changes in the gene can make it not work, which means the gene is knocked out. This method is better than homologous recombination, and so it can be used more easily to make biallelic knockouts.

Zinc-finger nucleases have parts that can attach to specific DNA and cut it in a precise way. Each zinc finger can identify specific parts of a DNA code, and can be put together to target a

specific sequence. These binding parts are connected to a restriction enzyme that can create a break in both strands of the DNA. Fixing things might change them and make them not work like before. The text is too short to rewrite in simple words. Can you provide a longer text for me to simplify. TALENS are a type of protein that can stick to a specific part of the DNA and cut it. The DNA binding area is made up of amino acid repeats. Each repeat can recognize one pair of bases in the DNA we want to target. If the cut is made in the middle of a gene, and repairs cause changes to the gene's code, it can cause the gene to stop working properly. CRISPR/Cas9 is a way to change genes in an organism. It uses a guide RNA and a protein called Cas9 to do this. The guide RNA can be designed to match a specific DNA sequence by pairing up complementary bases. This is faster and easier than building the constructs needed for zinc-fingers or TALENs. The joined Cas9 will make a break in the DNA with two strands. Just like zinc-fingers and TALENs, when trying to fix these breaks in a strand of DNA, it can cause mutations that make the gene not work properly. A conditional gene knockout means you can delete a gene from a specific tissue at a specific time. This is needed instead of removing a gene if it would cause the embryo to die. This is done by adding small sequences called loxP sites near the gene. These sequences will be added to the germ-line in the same way as a knock-out. This germ-line can be combined with another germ-line that has a viral enzyme called cre recombinase. This enzyme can find specific sequences, join them together, and remove the gene between those sequences. Knockouts are mostly used to study the effects of a particular gene or DNA area by comparing the knockout organism to a regular one with similar genes. Knockout organisms are used to help make new drugs. They can target specific parts of the body and help us understand how drugs work. For example, in Saccharomyces Cerevisiae, a library of knockout organisms helps us study the whole genome.

Biochemical signals usually come from liquid substances, which are either released by nearby cells or brought by blood vessels in the body. Insoluble ligands are sticky proteins or molecules like collagen, laminin, and carbohydrates. Chemical things usually affect the area around the cell based on how much of it there is. Chemical and biological methods are the best way to turn stem cells into different types of cells. Tiny particles, substances that help cells grow, and chemicals can have strong, far-reaching effects on the area where stem cells are found. Because they are easier to study, scientists know a lot about soluble biochemical cues and how they affect stem cells. This knowledge has been used a lot in lab experiments with stem cells. So, the next part will talk mostly about using different kinds of signals that can dissolve in water, like oxygen, and signals that don't dissolve, like certain chemicals and biological cues, to create specific environments for cells.

In the body, many growth factors and morphogens stick to the ECM by attaching to specific areas called heparin-binding domains or by connecting to collagen or cell membranes. "By trapping growth factors in this way, it can help the protein stay in one place and build up in high amounts, rather than spreading out or being taken in by cells. " For instance, the morphogen Sonic hedgehog (Shh) is changed at its ends by fats that attach it to the cell wall and keep it from moving around too much. Taking out the fats makes the factor less strong because it is not as concentrated. So, engineers are trying to copy the way cytokines naturally stick to cells to help them work better. This can make cells send signals more effectively and reduce the amount of growth factor needed for a strong response.

A study looked at a new idea for how to design something. They tried using epidermal growth factor (EGF), which is good for fixing damaged tissues. But it's hard to give EGF in high enough amounts to make it work well, because it doesn't stick to a matrix and gets taken up by cells quickly. In a recent study, scientists used a special method to attach a growth

factor called EGF to the surface of certain materials. The attached EGF made the EGF receptor keep working and caused cells to spread out and stay safe from cell death. But having a lot of loose EGF did not have the same effect. Sakiyama-Elbert and her colleagues Added heparin to the biomaterial to hold basic fibroblast growth factor in place. bFGF was given out by spreading out on its own or by being released through heparinases that nearby cells produced. This way, the signal was released slowly and controlled, which couldn't happen with just giving out the growth factor. The study used a delivery system to turn mouse stem cells into nerve cells, showing that materials with growth factors could be used to make tissue for treating spinal cord injuries. Recently, researchers found that certain materials with a special coating helped bone cells grow better than when they were exposed to the coating alone. This shows how keeping growth factors in place can help activate other processes for a longer time. This is because the amount of growth factor in one area is higher when it's stuck in place compared to when it's in a liquid form.

Surface modifications can be very useful in helping cells stick, grow, and change. This is especially true when we use protein biomolecules to give them more signals to do these things. The RGD sequence and some natural proteins like collagen, laminin, and fibronectin are really important for cells to stick to surfaces made of certain materials. Attaching these proteins to polymers helps cells stick and grow. It also makes the polymers more water-friendly. For example, it works with aliphatic polyesters. One way to modify biopolymer surfaces is by adding RGD peptides, which helps cells stick to synthetic polymer surfaces. This string of amino acids can connect with integrin receptors at specific attachment sites. Once the RGD sequence is found by the integrins, it will start a way for the cell to attach and send signals between the cell and ECM. This affects how the cell acts on the surface and can make it grow, change, survive, or move. The study found that by adding certain substances to the composite materials, it could help cells stick better and turn into bone cells. In a different study, they showed that stem cells in a certain type of gel can change into cartilage cells when a certain protein is present. More importantly, this means that the amount of RGD present in PEG hydrogels affects how well cells survive and grow cartilage.

While all the cells in our body have the same genetic material, they can become different kinds of cells with unique functions and shapes because of how the genes are organized and expressed. So, this means that by changing the way genes are turned on or off, any cell could potentially be changed into a different type of cell. The first report of cell reprogramming was mentioned in another report, where fibroblast cells were changed into myocyte cells by adding extra MyoD gene. In another study, the center of a fibroblast cell was put into an oocyte with no nucleus, and this made it possible for Dolly the sheep to be born. In 2006, Yamanaka found out how cells change and become different types by turning mouse cells into iPS cells. Being able to change specific cells to make them behave like certain types of cells could lead to many new ways to repair and heal the body. This could be a big breakthrough in making new tissues and medicines that help the body heal itself. In this study, making iPS cells from ordinary cells is a big deal because it can create versatile cells. The genome is not stable, it's expensive to grow, there's no good way to make it change into different types of cells, and when it's put into the body, it might cause tumors. These are the main reasons why it's taking a long time to use this in medicine.

Stem cells turning into different body parts is still a big problem in tissue engineering because the tissues and organs are so complex with many cells. To fix this problem, we need to use different kinds of cells for tissue engineering. This is just as important as copying the way the body works. Pluripotent stem cells have special abilities that allow them to renew themselves and turn into any type of cell. This is important for growing new tissues and

organs in the body. To create complicated tissues, we can follow the natural processes that happen inside the mass of cells in early embryos. This can help us make tissues that are connected and work together well. In a new study, scientists were able to make a 3D model of an organ for the first time ever. They were able to grow a structure similar to the optic cup with all six cell types found in a normal retina. They copied the way cells gather together and grow on their own to create a structure called an optic cup. This structure can produce cells for the retina, similar to how the eye forms in embryos. They used modified mouse cells with a specific DNA marker to study how the early stages of eye development happen. They wanted to see how the cells form and grow into eye tissue. Scientists want to use the same technologies that are used to make a 3D structure of the retina to make 3D structures of other organs like the brain, lung, and kidney. Although we have made progress, we still need to figure out if we can use pluripotent stem cells for regenerative therapy. It's not always best to make cells very strong and then weaken them to a low level by changing them. It's possible to directly change one type of cell into another type.

Studies have proven that fibroblast cells can be changed into different types of cells like muscle, brain, liver, and heart cells using direct reprogramming. This gives us good tools to create many different kinds of cells for regenerative medicine. New ways to change cells have been suggested.

This includes turning regular body cells into stem cells for specific tissues and changing fibroblast cells into stem cells for the brain. Cell reprogramming is good because it gives us three different types of cells that are needed for the brain and nervous system: astrocyte, oligodendrocyte, and neuron. Another benefit of using direct reprogramming to make tissue-specific stem cells instead of mature cells is that it provides all the needed cells to regenerate that specific tissue. For example, we have seen that turning fibroblast cells into NSC is better than turning them into neurons.

Additionally, when adult stem cells are created through direct reprogramming, they have the ability to keep making more of themselves, and can be set aside for future use in various medical treatments. Specific cells in the body called adult stem cells are naturally found in all tissues. These cells can move to where they are needed and respond to signals when the body is stressed or damaged. The human body is made up of many parts that work together and have special programs to control them. Using direct reprogramming, we can make progress in regenerative therapy if we have all the right materials, like adult stem cells, ES, and iPS, and we prepare them in the right way.

Adult stem cells, also called progenitor cells, are found in small amounts all over the body. They make specific cells for the area they are in. These cells do not regenerate as effectively as embryonic stem cells. However, if we put these cells in a new environment, they might create a different kind of cells than the ones they originally came from. Scientists are studying stem cells and learning new things about them all the time. For instance, new research shows that stem cells from blood may be able to make nerve cells. Researchers are still looking for new places to find adult stem cells. Stem cells have been found in bone marrow, skin, liver, blood, and the brain. Some grown-up stem cells, like those found in umbilical cord blood, have been used to help cure diseases. Blood-making cells, called hematopoietic stem cells, are found in the bone marrow. They create different types of blood cells. They have worked well to help young people with blood problems. Stem cells from the umbilical cord blood are in the cord's blood after a baby is born. Umbilical cord stem cells are like adult stem cells, but they are not as developed and can change into many different kinds of cells.

CONCLUSION

Many people have talked a lot about how stem cells could cure human illnesses, but there are still many challenges that need to be solved. We need to do more research on human pluripotent and multipotent cells because stem cell biology is different in humans compared to mice. Next, we need to understand better how cancer cells and pluripotent stem cells both can divide without limits. This will help us prevent the formation of cancer. Third, we need to learn how to get a lot of the right cells at the right stage of development. Fourth, we need to create specific plans to make transplanted cells grow, survive, and become part of the body. When it comes to stem cells, just knowing they exist is far from being able to use them to help people with medical problems.

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CHAPTER 12

RECOMBINANT VACCINES: UNDERSTANDING THE WAY OF TREATMENT

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ABSTRACT:

Vaccines were first made by trial and error, mainly by making germs weaker or inactive. New discoveries in the study of how the immune system works and how cells function have given us a better understanding of how vaccines work. Recombinant proteins help the immune system focus on a small number of important antigens. There are different ways to make proteins, and each way has its own strengths. This helps to produce a lot of proteins based on what's needed. Live modified bacteria or viral tools can trigger the immune system like a real infection and also have their own abilities to enhance the immune response. DNA vaccines are made of non-replicating plasmids and can create strong and long-lasting cellular immune responses. Prime-boost plans use different ways to give antigens to make the immune response stronger. Overall, all these strategies have their good and bad points. It depends on how well we understand how the pathogen causes infection and how the body's immune system responds in order to choose which strategy to use. In this article, we talk about important progress made with new types of vaccines. We also look at different ways to make vaccines and what problems they might have.

KEYWORDS:

DNA Technology, DNA Vaccine, Immune System, Immune Response, Recombinant Protein.

INTRODUCTION

Recombinant DNA technology is used to produce a recombinant vaccine. By putting the DNA with the germ's information into cells, we can help the immune system get stronger. After putting the antigen into these cells, getting it out from them. Recombinant vaccines are made by employing bacteria or yeast cells. A tiny bit of the virus or bacteria's DNA is taken and put into cells that make things. By mixing the body functions of one tiny organism and the genes of another, we can make the body better at fighting diseases with tricky ways of infecting us. A vaccine called RVSV-ZEBOV, made by Merck, is being used in 2018 to fight Ebola in Congo. The words vaccine and vaccination come from the term Variolae vaccine, which was used by Edward Jenner to describe cowpox when he made the first vaccine. In 1798, he used the phrase for the long title of his book "Inquiry into the VariolaeVaccinae Known as the Cow Pox". In the book, he talked about how cowpox can protect against smallpox [1]. In 1881, Louis Pasteur suggested that the terms be expanded to include the new vaccines that were being created to honor Jenner. The study of how vaccines are made and used is called vaccinology. A subunit vaccine is a vaccine that shows the immune system one or more antigens without putting in any harmful germs, either whole or in pieces. The word "Subunit" means that the antigen is a small piece of the germ, and the antigens can be any kind of molecule, like proteins, peptides, or sugars. Like inactive vaccines, this vaccine is completely "dead" and less risky [2].

Recombinant DNA technology is when scientists put DNA from different species together. The new DNA is put into another living thing to make helpful changes for things like medicine, science, farming, or business. The steps in making recombinant DNA can be summarized like this. The first thing to do is find the gene that makes the protein we're looking for. A gene that controls a good trait or protein needs to be found first. Some methods like Gene Chips and DNA sequencing can find the gene we want. The next step is to find and separate the gene we want from the species we are studying. This can be done by either smashing the cells or using special chemicals like detergents. The DNA can be separated from the rest of the cell using a method called cell centrifugation. To separate the gene, we want from all the other DNA, we need to follow these steps: First, we need to separate the DNA fragments by size using Gel Electrophoresis. Finding the gene, we are looking for using a special tool that detects DNA [3]. The gel is removed and then copied using PCR to make more of it. Instead, the gene we want can be put into a small circle of DNA in a bacterium using a special enzyme called DNA Ligase. Bacteria can copy genes when they divide, which is called Gene Cloning. If we have enough information about a gene, we can make special DNA primers to copy the gene using a method called PCR without isolating it on a gel. Then, we use a vector to put the gene into the organism we are changing [4].

The vector type depends on the type of cell. The final DNA sequence containing the target gene and its control sequences is called Gene Construct. But, it's hard to get the transgene to work well. In order for the target gene to work, it needs to get inside the nucleus. During cell division, the gene needs to become part of the new cell's DNA through recombination or crossing over. To check if the desired gene is in the genome, scientists add another gene called a reporter gene to the gene structure. This gene controls a trait that can be easily seen or chosen, such as being resistant to antibiotics or having a protein that glows in the dark. This allows scientists to easily check if the integrated gene is working or not. However, cells that have been changed genetically to make proteins need to be grown in a lot. To do this, 'Bioreactors' are used to make a lot of medicine like antibiotics and insulin [5]. A bioreactor is a round tool used for doing a biological or chemical reaction in specific conditions like heat, water, acidity, oxygen and stirring to make cells grow and be productive.

In the last few years, scientists have found a way to use DNA technology to make new and better vaccines. The different types of recombinant vaccines are subunit vaccines, attenuated recombinant vaccines, and vector recombinant vaccines. In this part, we will talk about different kinds of vaccines made by combining different parts of germs. These vaccines are very important parts of the harmful germs. "Subunit vaccines are made from parts of harmful germs such as small pieces of their proteins, DNA, or peptides. " Subunit recombinant vaccines are great because they are very pure, stable, and safe to use. However, subunit vaccines are costly and may change the original shape of the pathogen's part used to make the vaccines. Hepatitis B mostly affects the liver and can cause long-term liver problems and even liver cancer. The center of the Hepatitis B virus has its genetic material (DNA) surrounded by a layer of fat with surface markers. But, it is difficult to grow hepatitis B virus in cells and make surface antigens. The hepatitis B vaccine is made by putting a gene from the hepatitis B virus into yeast cells [6]. This helps create a vaccine to protect against hepatitis B. The HBsAg gene is put into the plasmids, and then the plasmids are moved and grown in a medium without tryptophan.

When the HBsAg gene is active, it makes very small particles that look like the ones found in people with hepatitis B. These tiny pieces can be separated to protect people from the dangerous hepatitis B virus. In these vaccines, harmful organisms like viruses and bacteria

are used. Organisms have been changed so they are not harmful in order to make vaccines. In the past, weaker forms of some very harmful germs were made by growing them for a long time. These weakened germs can't make you sick, but they can help your body build up its defenses. These days, scientists can change the genes of bacteria or viruses to make vaccines that are weaker and safer. Genetic alteration means either removing harmful genes from germs, or changing harmless organisms to show parts of harmful germs. The best thing about using attenuated vaccines is that they keep the antigens in their original form, which helps the body's immune system respond really well. Cholera is a sickness in the intestines caused by a germ. It causes fever, stomach pain, and being very thirsty. The current vaccines for preventing Cholera protect people for up to 6 months. So, scientists are trying to make better vaccines to fight the dangerous Cholera disease [7]. Through genetic engineering, scientists can now remove the DNA sequence that makes a toxin in the V bacteria. This creates a non-harmful strain of the bacteria. Cholera doesn't make enterotoxin. Genetically changed V. Cholera could be a good choice for making a weaker vaccine.

These vaccines are changed viruses that can fight against specific harmful organisms. Jenner first used vaccinia viruses to get rid of smallpox. This virus has a 187 kb DNA that makes about 200 proteins and can easily copy itself in the host cell. Foreign genes from a dangerous organism can be put into a virus to make proteins that can help fight off the disease. The best thing about using vaccinia virus to make vaccines is that it is safe, stays the same, and is easy to use. It can also help the body's immune system in two different ways. New vaccines have been made by mixing different parts of viruses.

These vaccines have worked well in preventing deadly diseases like herpes, flu, hepatitis, and rabies. However, these vaccines have not been tested on people yet because of safety worries. Recombinant DNA technology is used to make important substances like insulin and blood products. These man-made drugs can help people with genetic or lifestyle problems live better. In the next part, we will talk about how insulin and tissue plasminogen activator are made using a method called recombinant DNA technology. The first medicine made from cells of mammals was tissue plasminogen activator. It was made by Genentech in 1982. Tissue plasminogen activator is an enzyme that helps break down blood clots. Normally, plasmin breaks down fibrin and makes blood clots dissolve [8]. Tissue plasminogen activator is very helpful and can prevent blood clots in the heart and brain. Genentech made human insulin in 1978 using DNA technology.

Insulin is made of two parts, an A chain and a B chain, which are joined together by disulfide bonds. The first step in making insulin with recombinant DNA technology is to take the gene for insulin from the cells in the pancreas. The DNA of the bacteria is cut, forming an open ring. *E. coli* is a type of bacteria that can cause food poisoning and other infections. It is commonly found in the intestines of healthy people and animals. The *E. Coli* bacteria is often used to make human insulin, but yeast can also be used for this purpose. After that, the gene that makes the special protein is put into the open plasmid ring. Next, we put the DNA back into the bacteria we want to change. The changed bacteria are put into a good environment with all the things they need to stay alive and grow. When the bacteria multiply, they also make copies of the human insulin gene in the new cells. This process is done in a lab, and then it's done on a big scale to make a lot of insulin. Fermentation is the process of making important molecules in large amounts using science. To make a lot of insulin, we put cells that make insulin into big machines called fermenters. Lastly, the insulin released into the culture liquid is separated and made pure. This process includes separating cells from culture or broth, making the recombinant protein stronger, cleaning the recombinant product, and checking its quality.

DISCUSSION

Recombinant vaccines are usually made using bacteria, yeast, mammal, and insect cells. This vaccine needs to put the specific DNA part in the body to make the antigen. Out of the cells listed, bacterial expression is used most often and it doesn't need changes like mammal and insect cells do. Many of the new vaccines made by combining different parts are called recombinant protein vaccines. Recombinant protein vaccines are expensive and not easy to get. However, this type is much safer than others. In some vaccines, a weakened virus or bacteria is used to carry the medicine. These carriers make the immune system respond in the same way as if it were fighting a real infection. The tiny organism's genetic material connects to the carrier and activates the body's defense system. Another kind of recombinant vaccine is the DNA vaccine, also called a genetic vaccine. The DNA plasmid without any covering is put into the muscle using a needle or a "Gene Gun" machine. This vaccine can greatly activate T-cells and it is cheaper to purify than other similar vaccines. mRNA vaccines use a middleman called mRNA to help the body make proteins from DNA. mRNA vaccines are very strong, but some countries have limited their use because they are not very stable in tests with animals [9]. Right now, scientists are improving mRNA vaccines and need to study more to understand their role in fighting cancer and infectious diseases.

Most vaccines work well because they focus on germs that don't change much and need antibodies to protect against. This is about diseases like polio, tetanus, diphtheria, measles, and hepatitis B. Therefore, vaccines that can produce antibodies that can fight off these germs worked well. However, it is harder to develop vaccines that can protect against long-lasting infections caused by certain types of pathogens that attack the body's cells. The vaccines made from weakened germs can sometimes have dangers, like making people with weak immune systems sick, and possibly becoming harmful again. Recombinant vaccines work by using specific antigens to help the body build immunity to a virus or bacteria. They can be given with adjuvants or delivered by harmless bacteria or viruses. Recombinant protein vaccines are safer than vaccines made from purified macromolecules because they reduce the risk of having harmful substances mixed in or turning non-harmful substances into harmful ones. For example, this is important when making diphtheria or tetanus vaccines [10], [11]. This technology helps solve the problem of how hard it is to get enough purified antigenic components.

One of the main problems in making new vaccines is creating ones that activate the right immune response to protect against infections that live inside cells, and especially those that cause long-term illness. We need to understand the biology of certain antigens that cause disease and how the immune system protects us. This will help us design better vaccines that can fight infections more effectively. Many efforts have been made to find antigens that can protect us from diseases. Researchers have used different methods to choose the best antigens. However, using these antigens as vaccines is more than just finding them. Creating better vaccines will need different plans, like using various ways to deliver the vaccine and adding extra substances to help make the immune system respond well. These strategies will help the body fight against harmful things in a better way. Newer biotechnology tools have given us new ways to make vaccines. Here we look at some strategies that are being used now and talk about how they could be used to make new vaccines for people. We also talk about the problems that still need to be solved for these vaccines to be developed and used.

We have made copies of genes from different causes of diseases, put them into cells, and made them into clean substances to test as vaccines. There are different ways to make proteins that can trigger an immune response, like using bacteria, yeast, mammal cells, and insect cells. The DNA that makes the protein can be put into these cells and used to make the

protein. Before choosing the system for antigen expression, you need to consider a few things. The type of gene and promoter used, the selection marker, and whether the modified gene is changed after it's made are all important in making effective vaccines. Bacteria are commonly used for making proteins because they are easy to work with and can make a lot of protein. However, for antigens that need changes after being made, it's better to use mammal or insect cells [12], [13].

Many of the vaccines being studied now use purified proteins or parts of germs. Recombinant protein vaccines, like the hepatitis B vaccine, are used in humans as a classic example. Hepatitis B virus (HBV) infection is a long-lasting liver disease that happens all over the world. HBV really likes human liver cells because they have a special receptor on their surface that the virus can attach to. The vaccines we have now are made by putting the hepatitis B surface antigen (HBsAg) into yeast cells. The HBsAg makes virus-like particles in the body, which are good at making the immune system respond. This makes the HBV vaccine work well. The yeast system can release the antigen into the liquid around it, which makes it easier to purify. Additionally, yeast cells have some of the cellular tools needed to change proteins after they are made. This means they can add sugar molecules to proteins. The way the HBV vaccine is made has been given to different companies and now the price is lower because they are competing with each other. This means that most poor countries can afford the vaccine.

A newer type of vaccine called the recombinant vaccine has been developed for fighting against human papillomaviruses (HPVs). HPV is a very common disease that can be passed through sex. It can cause many different skin and mucous membrane diseases in people, like cervical, vulva, and vaginal cancers, and genital warts. Two vaccines are used to protect against HPV. They were made using VLPs from HPV-6, -11, -16, and/or -18 subtypes. These vaccines use L1 recombinant proteins from each subtype. These proteins are made in yeast or insect cells. The L1 is a big protein that makes up the outside shell of a virus, and when it is made in a lab, it forms virus-like particles (VLPs). The vaccines are given in three doses with a substance called aluminum potassium sulfate that helps produce strong virus-fighting antibodies in the blood. These vaccines belong to the company and cost a lot of money. Because of this, it will be hard for poor countries to get them for a while.

Although vaccines made from recombinant proteins have many benefits like being safe and cheap to produce, a lot of them don't work well on their own. So, they need adjuvants to help them create a strong and long-lasting immune response. Recombinant proteins like hepatitis B and HPV have been used successfully for vaccines. This was possible with the help of aluminium salt as an adjuvant. So, finding new adjuvants for vaccines is very important in the study of vaccines. The biggest challenges in making new adjuvants are understanding how they work at a molecular level and how they help the immune system. For instance, we don't know how aluminum salts, which are often used in vaccines for people and animals, work. But, Richard Flavell's team recently said that they found a way to turn on a natural immune system inside cells called Nalp3 inflammasome. One other way to show antigens to the immune system is by using live bacteria or viruses, and studying how their natural properties can help boost the immune response [14], [15]. Creating and making sure a product is safe are also important things to think about.

Due to progress in science, we can now make special live cells that can carry different antigens by putting in genes that code for them. The reason for this method is to use a live vector's ability to cause infection and immune system properties to trigger a response against its own proteins and a different protein. Many bacteria and viruses have been studied to see if they can be used as vaccines. Usually, these methods have benefits that come from the virus

itself, like imitating a real infection, being able to activate certain types of T-cells, and sometimes being able to be taken by mouth. Live-attenuated bacterial vaccines have been used for a long time. However, using them to carry or deliver different antigens can be very helpful for making vaccines. This technology can be used to make a big difference in vaccine development. Big improvements in molecular biology have allowed scientists to carefully remove genes that make harmful things in the body, and add new DNA to vaccines to make them better at fighting diseases. Bacterial vectors are good for showing different antigens because they have a lot of benefits. They can trigger the body's immune system and can be taken by mouth to trigger immunity in the mucous membranes. Many bacteria can be killed by antibiotics, so they can be used to treat infections if there are side effects. Usually, they are very cost-effective.

Among these bacterial vectors, M. bovis BCG and S. typhi are the most emblematic of the current state of this method, as shown by the many and diverse publications published with both vectors (18,23). BCG has various characteristics that make it an ideal vaccination vector. It is safe and has been supplied to over 3 billion people with no adverse effects; it may be given shortly after birth; it is an effective adjuvant; and it has the potential to generate T cell-mediated immunity against the cloned heterologous antigen. This final trait is thought to be critical to an effective vaccination against intracellular infections. Several instances of recombinant BCG (rBCG) expressing foreign antigens from various infections have been reported, including malaria, tuberculosis (TB), HIV, leishmania, pertussis, and others. These have been shown to produce both humoral and cellular immunological responses, as well as protection against infection. Various strains of rBCG-HIV have been shown to produce both humoral and cellular immune responses against HIV antigens, including eliciting specific antibodies, producing interferon (IFN)-γ, and inducing T helper cells and cytotoxic T lymphocytes. Another intriguing finding is rBCG expressing the non-toxic subunit 1 of pertussis toxin (rBCG-S1PT). This strain was found to activate a cellular immunological response in adult and neonatal mice, protecting them against a fatal challenge with virulent B. pertussis. An antibiotic-free strain has been developed using autotrophic complementation and will be tested in clinical trials.

Recently, numerous studies have focused on the use of rBCG to increase TB protection. Recombinant BCG expressing essential M. tuberculosis antigens, such as Ag85A, has been found to stimulate greater immune responses than regular BCG in animal models, and as a result, these strains are being tested in human trials. In fact, rBCG-Ag85A was the first rBCG vaccination to be tested in a clinical study for tuberculosis. The goal was to enhance the BCG vaccination by overexpressing an immunodominant antigen that has been shown to be protective. Another BCG-based vaccination in clinical trials uses a more advanced technological method. In this situation, a BCG mutant lacking the urease gene was utilized to express the Listeriolysin O gene from L. monocytogenes. Listeriolysin expression in this vaccination may cause phagosome membrane breakdown, enabling BCG antigens to escape into the cytosol and thus boosting CD8+ T cell presentation and protection [16], [17]. rBCG strains expressing antimycobacterial cytokines, such as IFN-γ and IL-2, have been used to enhance the immune response against tuberculosis. However, concerns about potential cytotoxicity have been raised. TB protection relies on a Th1 immune response and IFN-γ production by particular T cells. However, there is no link between IFN-γ levels and the degree of protection.

The direct injection of a naked DNA plasmid into muscle as a vaccination system capable of eliciting an immune response and providing protection following challenge is now well established, having been utilized to produce several antigens from various diseases with

encouraging results. A DNA vaccine is made up of a plasmid that contains: one Escherichia coli replication origin for plasmid amplification; a strong promoter, typically from cytomegalovirus; multiple cloning sites for inserting the gene to be expressed; and an antibiotic as a selection marker. The concept behind the DNA vaccination method is that the antigen may be produced directly by the host's cells, similar to what happens during viral infection. As a consequence, antigens may be digested into proteins generated in the cytoplasm, and the fragmented peptides are given to the immune system via class I MHC molecules. Furthermore, if the protein is exported or secreted, class II MHC molecules may process it, resulting in a particular antibody response.

Initially, DNA vaccines were administered by intramuscular injection or using a DNA particle delivery method known as Gene Gun. Unlike intramuscular injection, which takes micrograms of plasmid DNA and several doses, the Gene Gun technique just requires nanograms of plasmid DNA to provide the same amount of immunological response. However, the two systems produced diverse immune responses to the same antigen. While im injection elicited mostly a Th1 response, Gene Gun vaccination elicited a mixed Th1/Th2 or Th2-shifted phenotype. These results are especially relevant in vaccine design since it is desired to know ahead of time what kind of immune response is necessary for protection against a certain disease. DNA vaccines have several properties that may give them an advantage over other immunization procedures: there is no risk of infection, unlike attenuated vaccines; they elicit both humoral and cell-mediated immunity; and they can induce long-lived immune responses as well as increased cytotoxic T-cell responses. Furthermore, DNA vaccines circumvent issues associated with recombinant protein vaccine production, such as insufficient target molecular folding or expensive recombinant protein purification costs. Although DNA vaccines have many advantages, some concerns about their suitability and capability should be investigated, such as the possibility of producing anti-DNA antibodies, integrating DNA plasmids into the cell genome and low cell transfection efficiency in vivo.

DNA vaccines have been utilized to express antigens from a variety of diseases, including influenza, HIV, malaria, tuberculosis, and leishmaniasis, inducing immune responses against these etiologic agents in numerous animal models and, in some instances, providing protection. However, DNA vaccines have been proven to be less immunogenic in both non-human primates and humans, while being safe and well tolerated. To improve the efficacy of these vaccines, a second generation of DNA vaccines has been developed. Some of these new strategies include plasmid alterations that enhance gene expression and systems that co-express cytokines or other molecules capable of enhancing immune responses. Co-expression molecules include genes that cause apoptosis and genes that encode ligands for Toll-like receptors (TLRs).

Other major technologies that have been discovered include the formulation of DNA in ways that prevent degradation or enhance its absorption into cells. One such example is DNA encapsulation in microparticles, or the use of living vectors like viruses or bacteria to protect and promote DNA transport into particular cells. In vivo electroporation, a method that uses modest amounts of electric current to produce the localized development of holes in cells, may also increase DNA absorption into cells. However, the widespread use of electroporation in vaccination campaigns is difficult to imagine. Despite the relative effectiveness of DNA vaccines in boosting immunogenicity, the specific biological processes by which they function in the body remain unknown. Again, since DNA vaccines alone have been demonstrated to be insufficient to elicit a robust immune response, tactics such as prime boost have been employed to enhance the immune response in order to build effective vaccinations against a number of infectious illnesses.

CONCLUSION

Recombinant vaccines are made from both DNA and proteins. The DNA version is less expensive to produce and can withstand high temperatures, so it doesn't need to be kept cold during transportation and storage. Many studies have been conducted to create DNA vaccines. Live recombinant vaccines are created using a weakened form of a virus or bacteria. This form is used to carry the genes that make the vaccine antigens. Live recombinant vaccines have many good qualities. They can help the body make both antibodies and cell immunity.

However, big improvements in the study of how the immune system works, how DNA is changed, small organisms, genes, and other related areas have given us new knowledge to understand how infectious diseases work and how germs interact with the body. All of these improvements have helped create many new ways to make vaccines that are showing good results. It is now clear that we need to use a combined approach to keep making progress in the field of immunology. This is important because it's probably the main thing holding us back from developing new vaccines.

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CHAPTER 13

GENETIC ENGINEERING TRANSGENICS: EXPLORING THE WORLD OF GENE MODIFICATION IN ORGANISM

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ABSTRACT:

Genetically engineered organisms, including plants, animals, or tiny organisms, are labeled as genetically modified organisms or GMOs. When genes from one type of living thing are put into another type, the new living thing is called transgenic. GMOs are organisms that have been changed by scientists in a lab to make their DNA different. Genetic modification alters the genetic makeup of plants and animals by blending genes through mating. In the next part of this module, we will explain more about how scientists make genetically modified crops. Rewrite this text using simpler words. GMOs can range in size from microorganisms like yeast and bacteria to larger organisms such as insects, plants, fish, and animals. Genetically modified crops are plants that have been changed to have new characteristics. Genetically modified crops are designed to withstand pests, diseases, and challenging environmental factors. They can also resist chemicals that farmers use to protect their crops. The plant is not affected by the weed killer. Genetic modification of crops can also be done to make them more nutritious, like how golden rice was made. Many people are talking about whether it's a good idea to use GM crops. Right now, it's believed that eating genetically modified foods is not harmful. Genetically engineered foods are created and marketed with the belief that they offer advantages for either the manufacturer or the buyer. This is supposed to turn into a cheaper product, lasts longer or is healthier, or all of these. Currently, the GM foods for sale worldwide have been checked for safety and are not expected to cause any harm to people's health. Additionally, there is no evidence to suggest that these foods are harmful to the health of individuals when consumed by the general population in countries where they are permitted.

KEYWORDS:

Cells, DNA, Gene, Genetic engineering, Plant Cells.

INTRODUCTION

Genetic engineering is a type of science that changes the genes of an organism using different tools and techniques. Transgenics means taking genes from one type of animal or plant and putting them into a different type. Transgenic organisms can use 'Trans' genes well because their genetic sequences for proteins are very similar to those of other species. Genetic engineering is when scientists change the genes of an organism using technology. It is some technologies used to change the genes of cells, including moving genes between different species, to make better or new organisms. New DNA is made by taking the genetic material and either copying it or putting it together using special methods. This can be done by isolating the DNA, copying it, or making it from scratch. A construct is made and used to put this DNA into the host organism. In 1972, Paul Berg made the first recombinant DNA molecule by mixing DNA from the monkey virus SV40 with the lambda virus [1], [2]. The

process can also be used to take genes out, or "knock out" genes. The new DNA can be put into the genome in a specific place or in any place. Genetic engineering has been used in many areas like research, medicine, making things and farming. In science, GMOs are used to learn how genes work and show their activity by turning them off, turning them on, following them, and studying how they are used. By removing genes that cause certain conditions, researchers can make animals that have the same diseases as humans. Genetic engineering can make hormones, vaccines, and medicines. It also can treat genetic diseases using gene therapy. The methods used to make drugs can also be used to make things like enzymes for laundry detergent, cheeses, and other products [3].

Genetic engineering is when scientists change an organism's genes using technology. Genetic engineering is a way to change the genes in cells to create new and better plants or animals. This can involve moving genes between different species. New DNA is made by copying the genetic material using special methods or by creating it in a lab. A construct is made and used to put this DNA into the host organism. In 1972, Paul Berg made the first recombinant DNA molecule by putting together DNA from the SV40 monkey virus and the lambda virus. In addition to adding genes, the process can also be used to get rid of genes. The new DNA can be put in anywhere or in a specific part of the genetic code. A living thing made by changing its genes is known as a genetically modified organism (GMO). Herbert Boyer and Stanley Cohen made the first GMO bacterium in 1973. Rudolf Jaenisch made the first genetically modified animal by putting new DNA into a mouse in 1974. In 1976, the first company to work on genetic engineering, Genentech, was started. They began making human proteins [4], [5]. In 1978, scientists made human insulin by changing its genes. In 1982, they started selling bacteria that can make insulin. Genetically changed food has been available since 1994, when the FlavrSavr tomato was first introduced. The FlavrSavr was made to last longer without going bad. But today, most genetically modified crops are changed to be able to survive against bugs and weed-killing chemicals. The first genetically modified pet, called GloFish, was sold in the United States in December 2003. In 2016, salmon that were changed with a hormone to help them grow bigger were put up for sale.

Genetic engineering has been used in various areas like research, medicine, making products, and farming. In research, scientists use GMOs to study how genes work and are expressed by turning them off, turning them on, and tracking how they work. We can make animals that have the same diseases as humans by changing their genes. Genetic modification has the potential to create hormones, vaccines, and other pharmaceuticals. It also has the potential to treat genetic diseases through gene therapy. The methods used to make drugs can also be used to make things like enzymes for laundry detergent, cheeses, and other products. Genetically modified crops have helped farmers make more money in many countries, but also caused a lot of arguments about the technology. This has been around since it was first used; the initial tests were ruined by people who are against genetically modified crops. Even though most scientists say that genetically modified (GM) food is just as safe as regular food, some people are still worried about its safety. Movement of genes, effects on other animals and plants, control of the food we eat, and who owns the ideas are all things people are worried about [6], [7]. These worries made the government create rules, which began in 1975. It caused a worldwide agreement called the Cartagena Protocol on Biosafety to be made in 2000. Different countries have made their own rules about GMOs. The US and Europe have the biggest differences in their rules.

Scientific advancements have enabled us to identify and manipulate genes. This led to the start of biotechnology, which has been around for over 20 years. New biotechnology tools are helping scientists solve problems in the life sciences. Agriculture is also changing because of

these tools. The rapid advancements in biotechnology are challenging our society, as well as agricultural research and education, to stay current. We have difficult choices and problems now, and we will have more in the future. Competition from other countries makes us come up with and use new technologies to make Indian farming better and more efficient. We should focus on making life better for all our people so that everyone can live with respect and happiness. This deserves our full attention in our growth plans. This means that when we achieve the goal of everyone having enough food, it's a natural result. The amount of land available per person is getting smaller because there are more people. This problem has been made worse by more wasteland. Urban areas are growing and people want more land. We need to grow more food on the small amount of land we have to make sure everyone has enough to eat. However, we need to remember that when making food, we should make sure that we don't harm the environment.

Biotechnology is now real and possible. Biotechnology is the use of technology based on living things to create products. It includes methods like gene transfer, cell culture, and engineering processes. We started turning ideas into things we can actually use by using these methods. For example, scientists have figured out how to change the genes of some plants so they can survive better against certain types of harmful germs like bacteria, viruses, and fungi. We have only just started to understand all the good things that biotechnology tools can do. Biotechnology gives new ideas and methods that can be used in farming. It helps us understand how living things work, our surroundings, and ourselves better. However, making more progress will require a lot of skilled people and money. Biotechnology can help make crops grow better. It can help scientists find new ways to make plants strong against diseases and cut down on using expensive chemicals for farming.

DISCUSSION

Genetic modification involves altering an organism's genes using technology to enhance its characteristics. The process of genetic engineering frequently includes incorporating new DNA from different organisms through a technique known as molecular cloning. The organism that receives the new DNA is known as a genetically modified organism (GMO). An organism is considered transgenic if its DNA comes from a different species. Since the early 1970s, scientists have changed the genes of bacteria, plants, and animals for learning, medicine, farming, and industry. Roundup-ready soybeans and borer-resistant corn, both GMOs, are commonly found in processed foods in the US. Classical ways to study how genes work used to start with how something looks and then figure out what genes make it look that way. Nowadays, researchers can begin their work by examining the DNA sequence and inquiring about the function of a specific gene or DNA segment." This new way, called reverse genetics, has changed how genetic research is done. This approach is akin to causing harm to a body part in order to understand its function. An insect that loses a wing cannot fly because the wing is used for flying. The old-fashioned way of studying genetics would look at bugs that can't fly and bugs that can, and see that the ones that can't fly have no wings [8], [9]. In the same way, changing or removing genes helps scientists understand what the genes do. Gene targeting refers to the different ways that are used to stop genes from working. Gene targeting is when scientists use special DNA tools to change how a specific gene works in an organism. This can be done by making changes to the gene or removing it completely from the organism's genetic material.

Genetic diagnosis is when doctors test a person's genes to see if there are any problems before they start treatment. Family members should get genetic testing to find out if they have a gene that causes a disease. This depends on how the gene is passed down in the family. For instance, when women are told they have breast cancer, doctors usually suggest getting a

biopsy to find out what is causing the cancer. The treatment options are determined based on the findings of genetic tests that identify the specific type of cancer an individual has. If there is a genetic predisposition to cancer within the family, it is crucial for other female relatives to be screened for the gene and undergo regular breast cancer examinations. Genetic testing can also be done for fetuses or embryos made through in vitro fertilization to find out if they have genes that can cause certain diseases in families. Gene therapy is a way to fix illness by changing genes. In simpler terms, gene therapy is when a healthy gene is put into the body to help treat a disease caused by a problem with a gene. The healthy gene is put into sick cells using a virus that can infect the cell and carry the new DNA. More advanced gene therapy tries to fix the mistake in the genes where it first happened, like when treating severe combined immunodeficiency (SCID).

Old-fashioned vaccines use weakened or inactive germs to help the body build up its immunity. New methods use the genes of tiny living things put into carriers to make a lot of the needed substance. The antigen is put into the body to make the immune system respond and remember it for the future. Genes taken from the flu virus have been used to fight the different kinds of this virus that keep changing. Antibiotics are a type of medicine made using biotechnology. Microorganisms like fungi make these things naturally to beat bacteria. The process of growing and modifying fungal cells is used to create a large supply of antibiotics. Recombinant DNA technology was used to make a lot of human insulin in bacteria. Coli was found as early as 1978. Before, people could only use pig insulin to treat diabetes. But this caused allergic reactions in humans because of genetic differences [10], [11]. Right now, most people with diabetes who use insulin injections get it from bacteria. Human growth hormone (HGH) is medicine for helping kids who are not growing as they should. The HGH gene was copied from a cDNA library and put into E. Bacteria are used to make copies of the gene by putting it into a bacterial carrier. Bacteria HGH can help people with growth problems feel better. When it comes to creating recombinant proteins for medical use, bacteria are the typical choice, however, some proteins necessitate an animal host for proper production. Because of this, scientists copy and show the genes they want in animals like sheep, goats, chickens, and mice. Transgenic animals are animals that have been changed to have new DNA. Certain proteins produced by humans can be found in the milk of specific sheep and goats, as well as in the eggs of chickens. Mice have been used a lot to see how different genes and changes in genes affect them.

Changing the DNA of plants, known as GMOs, has made plants stronger against diseases and weed killers. It has also made them more nutritious and last longer. Plants are very important for people because they provide most of the food we eat. Farmers discovered techniques to choose plants with favorable characteristics prior to the use of modern biotechnology. Transgenic plants are those that have been genetically modified with genes from different species. The government keeps a close eye on transgenic plants and other GMOs to make sure they are safe to eat and won't harm other plants and animals. We need to do a lot of testing to make sure that foreign genes don't spread to other animals and plants in the environment and cause problems. Basic crops like corn, potatoes, and tomatoes were among the first plants to be changed through genetics. Microorganisms can share genes with each other from different species. Many viruses can cause diseases in humans, like cancer, by putting their DNA into the human genome [12], [13]. In plants, a disease called tumors can be caused by a type of bacteria called Agrobacterium tumefaciens. This happens when the bacteria's DNA is transferred to the plant. Even though the tumors don't directly kill the plants, they make the plants smaller and more likely to be harmed by tough surroundings. Some plants like walnuts, grapes, nut trees, and beets get sick because of Agrobacterium tumefaciens.

Agrobacterium tumefaciens is a type of bacteria. It's harder to put DNA into plant cells than animal cells because the plant cell wall is thick. Scientists used a method to put DNA from one organism into a plant to add specific DNA pieces to the plant. In the wild, the A-causing disease. The tumefaciens have some plasmids called Ti plasmids that can make plants grow tumors. The DNA from the Ti plasmid goes into the plant cell's genes when it gets infected. Scientists change the Ti plasmids to take out the genes that cause tumors and put in the DNA they want to transfer into the plant's genes. The Ti plasmids have genes that make bacteria resistant to antibiotics. They can be grown in E. Coli cells are also affected. Bacillus thuringiensis (Bt) is a kind of bacteria that makes poisonous protein crystals. These crystals harm insects that harm plants. Insects have to eat Bt toxin for it to work. Insects that have eaten Bt toxin stop eating the plants within a few hours. After the poison is turned on in the bugs' stomachs, they die in a few days. Modern biotechnology has enabled plants to make their own toxin called Bt that kills insects. The crystal toxin genes from Bt were copied and put into plants. Bt toxin is okay for the environment, not harmful to people and animals, and is allowed for use by organic farmers to kill bugs. The first genetically modified crop sold in stores was the FlavrSavr Tomato, made in 1994. We used a method called antisense RNA to make the GM tomatoes last longer by preventing them from getting soft and rotting because of fungal infections. The taste of this tomato got better with more genetic changes [14], [15]. The FlavrSavr tomato didn't stay in the market because it had problems being kept fresh and shipped to stores.

The practice of incorporating and utilizing genes from different plants in plant cells has become widespread in numerous laboratories. It helps scientists' study how genes work and create new kinds of plants that could be useful for farming. Plants can now have different genes from other countries put into them. Originally, this was accomplished using tobacco, but now it has been expanded to include numerous other plants. Genetically modified crops like tomato, papaya, cotton, maize, and soybean are ready for people to eat. They will help improve how we grow food and have a big impact on making more food, growing the economy, and having a better way to farm in the future. Even though being able to change genes in plants is helpful for science, a lot of focus on this research is because it creates plants with traits that breeding can't create, like being resistant to viruses, insects, herbicides, or spoiling after being harvested. This helps with practical things and studying plants. This chapter explains the technical details of the latest advances in plant engineering. It also finds problems with the technical side of developing systems to transform plants for improving crops.

This bacterium can change normal plant cells into tumor cells. Plant biologists use Agrobacterium to change the cells of plants by adding a piece of DNA. This helps them change the genes of plants, which is important in plant science. This natural way of moving genes is really good at it and often results in cells that have only one copy of the new DNA. It's simpler compared to other ways of changing genes. The tool was made by learning how crown gall disease works. This disease happens when a bacterium transfers DNA to a plant, causing a tumor to form. Just a small and separate part of the area. A tumor-causing plasmid (Ti) is moved from a bacterium to a plant's genes. The T-DNA is a piece of DNA that has two sets of repeating letters and contains genes that help plants grow and produce certain chemicals. Bacterial proteins called vir genes help transfer DNA from the Ti-plasmid to the plant. These genes are switched on when the plant tissue is damaged and releases certain chemicals. The important thing to know about gene transfer is that the T-DNA genes are not directly involved in the transfer process. This means that we can change or remove these genes and still transfer the T-DNA region to the plant's genes.

For a while, people thought that Agrobacterium tumefaciens could transfer genes to any type of plant. Since this did not happen, many different ways of transferring genes directly have been tried. Many ways to put genes directly into an organism, like using electricity to get DNA into cells. Taking DNA into cells with the help of PEG, fusing protoplasts with liposomes containing DNA, using biolistics or microinjection, all need plants to regrow from protoplasts. Many plants are not good at growing back from protoplasts, and it takes a long time to grow them in tissue culture, which is a problem. Other ways to put genes directly into plants or tissue do not need protoplasts. Biolistics is a technique that uses heavy microparticles covered with DNA to carry genes into different cells and tissues in the body [16], [17]. This method is good because it is easy to use and can create many different types of cells at once without needing a lot of work. It can be used on a wide variety of cells like pollen, cultured cells, and meristematic cells. This method has been used to create many genetically modified crops. Electroporation is a common way to easily change plants from protoplasts. This method involves using a strong electric field to make bilipid membranes permeable, and it can work on all kinds of cells. When a capacitor releases its stored energy, it creates temporary openings in the cell wall. This allows DNA to enter the cells if the DNA is touching the wall directly. Plants made using this method have a few copies of the altered DNA, which is usually passed down from one generation to the next in a predictable way.

It is possible to introduce DNA into a wide range of plant cells for regeneration through the use of Agrobacterium or direct gene transfer techniques. However, only a small number of the treated cells change while most of the cells stay the same. It is important to find changed cells from a lot of unchanged cells and to create conditions that help to grow healthy plants from the changed cells. "Selectable marker genes are needed to add important genes to crop plants that are important for farming. " We always put the agronomic gene we're interested in together with marker genes. Only the cells that have and use the marker gene will stay alive in the lab. Plants grown from the cells that survived will have a marker connected to the important gene for farming. Usually, we choose transgenic plant cells by putting in a gene that makes them resistant to antibiotics or weed killers. Then we can pick out the transgenic cells by putting them in a solution that contains the toxic compound. The antibiotics and herbicides are special ingredients that are used only in the lab at the beginning of the process to choose cells that have the genes for certain farming traits. The chemicals are not used after the new plants grow from those cells and as the crop continues to grow in the field. So, these plants and all the other plants and plant products that come after them will not have been in contact with, or contain, the selective agent. The most commonly used gene for making choices is called neomycin phosphotransferase II (NPTII). This gene makes cells resistant to certain antibiotics. Other selective systems have been made using resistance to bleomycin, bromoxynil, and chloramphenicol.

Reporter genes are like labels that can be used to see which cells have been changed and to study how genes are turned on and off. In addition, reporter genes are helpful for finding genetic changes. They don't help transformed cells live in certain lab conditions. They just find or label the transformed cells. Genetically modified plants require more than one cell for growth, making their direct selection ineffective. This makes it important to find alternative methods for their cultivation. When it's not feasible to directly pick genetically modified plants, alternative methods become crucial for their growth. It is essential to find alternative methods for the growth of genetically modified plants, as they cannot be effectively grown from just one cell and direct selection is not practical. They can also be useful in measuring how well a gene has been transferred and how much it is being used in the transformed cells. The reporter gene used in plants should not show much activity in the background, should not harm the plant's metabolism, and should come with an easy, cheap, and versatile test that can

measure its activity accurately. The gene for the enzyme glucuronidase, GUS, has been made for showing changes in plants. The enzyme GUS breaks down a lot of different substances and can be used in tests to see changes in plants. GUS has many helpful features that make it a great reporter gene for studying plants.

First, many plants that have been tested so far do not show any GUS activity, which means we don't have a starting point to test the expression of chimeric genes. Secondly, glucuronidase can be measured easily and at a low cost, both in the lab and in gels. It is also strong enough to withstand being fixed, which allows it to be located in cells and tissue sections using a special staining method. Thirdly, the enzyme can handle big additions at the beginning of amino acids, which allows making combinations during translation. The gene that makes firefly luciferase is very good for showing how well enzymes are working. It is sensitive, quick, easy to do, and not very expensive. Luciferase makes light really well compared to other reactions. Also, luciferase is a single protein that doesn't need any extra processing to work as an enzyme. Scientists use a glowing protein from jellyfish to tag plant cells. This helps them track how genes are working inside the plants as it happens. GFP glows green when you shine UV light on it. GFP does not need anything else to work, unlike other reporters. GFP stays the same, works for all kinds of living things, and can be checked without hurting the cells. It lets us see the glowing gene product in living cells without having to use long and harmful staining methods. Also, it's easy to see if GFP is being produced in plants by using a long-wave UV lamp, as long as the fluorescence is strong enough. Another good thing about GFP is that it's small and can be attached to the beginning or end of a protein. This makes it useful for studying where proteins are in cells and how they move around. It has been found that too much GFP can harm how plants grow and develop. The way to solve this problem is by using mutant genes of GFP. Of all the changes in GFP, the S65T mutation makes it glow the brightest and it forms faster and doesn't fade as easily as regular GFP. Also, this mutant has one peak of light that works well with certain filters and it does not harm the plant cell.

Transgenics refers to the technique of moving genetic material from one organism to another, such as from a plant to an animal. A transgenic organism is an organism that has a gene added to it in a lab, instead of through natural breeding. Hybrids are created when cells from two different species come together to make a new organism. For example, the mule is the best example seen in nature. It is made when a horse and a donkey have a baby together. Moreover, in labs, chimeras are created by mixing genetic material from two different organisms to make one species. First, we find the gene that makes the protein we want. First, we need to find the gene that makes a good trait or protein. We can use techniques like Gene Chips and DNA sequencing to find the gene we want. The next step is to find and separate the gene we want from the organism we are studying. It can be done by either physically breaking the cells or using chemical substances like detergents. The whole DNA can be taken out from other parts of the cell using a method called cell centrifugation. To separate the gene, we want from all the DNA, Sort the DNA fragments by size using Gel Electrophoresis. Finding the gene, we want by using a special DNA tool. The gel is cut and then copied using PCR. Instead, the gene we want can be put into a bacterial plasmid using a special enzyme called DNA Ligase. Bacteria can make exact copies of a gene when they divide, which is known as Gene Cloning. If we have a lot of information about the gene we want, we can make special DNA tools called primers and copy the gene using PCR without separating it on a gel.

A special tool is used to put the desired gene into the organism. The tool type changes depending on the cell. The Gene Construct is the finished DNA sequence with the target gene

and its control parts, like the promoter and termination sequences. But it is very hard for the transgene to be expressed successfully. The target gene needs to get into the nucleus in order to be activated. During cell division (mitosis and meiosis), the gene needs to become part of the target cell's DNA through recombination/crossing over in order to be inherited. Scientists add a second gene called a reporter gene to the gene they want to study to check if it has been put into the genome. This gene controls a trait that is easy to see or pick out, like being resistant to antibiotics or having a protein that glows in the dark. This helps researchers check if a gene is working or not. Genetic engineering is using different methods to change an organism's genes. Transgenics helps scientists make organisms that have a new trait that they don't have naturally. Next, we will talk about new things happening in the transgenics field. Transgenics is about making new organisms that have a trait the original species doesn't have. For example, making potatoes that have lots of protein or rice that has a lot of vitamin A, also called golden rice.

Studying and developing transgenic technology could help protect endangered animals from deadly diseases. Genetic mixtures could be used by scientists to create vaccines for very dangerous illnesses. For example, scientists put the DNA of human cancer cells into tobacco plants to make a vaccine for non-Hodgkin’s lymphoma. Researchers have also made a flu vaccine using human DNA and tobacco plants. Genetically modified plants have been used to make vaccines that can be eaten and are safe for people.

These vaccines can help protect against diseases like cholera, hepatitis B, and rotavirus. Scientists put a gene for a human protein into fruits like tomatoes and bananas to make these vaccines. The Glowing Plant Project wants to put a firefly gene into a houseplant to make it glow in the dark. Another idea is to make plants that can glow and light up streets and paths. This will help save energy. Other uses for this technology could be in cars, airplanes, and protecting military people. Xenotransplantation is when tissues or organs from one animal are put into another animal. It could help more people get the organs they need, like hearts and kidneys. Pigs have a similar body and organ size to humans, so they are good for transplanting organs into people. Scientists are finding that transplanting cells can help people with serious diseases like Parkinson's or spinal cord injuries. Changing the genes of stem cells and growing them on a frame can be used as temporary skin to heal wounds or burns. Tissue engineering is quickly becoming a good way to replace parts of the body like cerebrospinal shunts, heart valves, cartilage, and other organs. Companies are spending a lot of money on research to make medicines from the milk of genetically modified animals like rabbits, goats, and cows. These medicines can help treat diseases like cancer, arthritis, and other immune system disorders. In conclusion, transgenics can help patients and doctors treat many types of disorders.

CONCLUSION

Even though we have learned a lot about how genes in plants and their diseases work, there is still more to discover. It is not clear if people all over the world, including India, will accept the use of genetically modified plants. Whether these methods will have a significant impact on the ongoing battle between plants and diseases remains uncertain. Stronger ways of controlling diseases can kill off some germs, but it might also cause super strong germs to grow that need even stronger controls. Similarly, not getting involved can cause problems too. Besides producing less crops, many molds create toxic substances. If we don't control these toxins well, the crops can be even more dangerous for humans than if they have pesticide residue.

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