Nigerian Journal of Pharmaceutical and Applied Science Research,
Vol.14 (1): 30-45; March 2025 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v14-i1-582

Transgenic Animals: The promise and peril of genetic modifications

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ABSTRACT

***Background**:* Transgenic animals, developed through genetic engineering techniques, have revolutionized biomedical research, agriculture, and pharmaceutical production. By introducing foreign DNA into animal genomes, scientists have enhanced traits such as disease resistance, improved food production, and enabled the generation of biopharmaceuticals. Despite these advancements, concerns regarding ethical implications, biosafety, and environmental risks remain. This review explores the methodologies, applications, and potential risks associated with transgenic animal technology.

***Methods**:* A comprehensive literature review was conducted, analyzing peer-reviewed articles and experimental studies on transgenic animal technology. Key methods used in generating transgenic animals, including vector-mediated gene transfer, DNA microinjection, sperm-mediated gene transfer, testis-mediated gene transfer, and somatic cell nuclear transfer (SCNT), were critically examined.

***Results**:* Transgenic animals have yielded significant advancements in medicine, including the production of recombinant proteins, disease models, and potential organ donors for xenotransplantation. In agriculture, genetic modifications have improved livestock productivity, enhanced nutritional value, and contributed to food security. However, the peril of these genetic modifications of animals, such as unintended genetic mutations and potential biodiversity loss, necessitate stringent biosafety regulations.

***Conclusion**:* While transgenic animals hold immense promise in scientific and medical advancements, their applications must be balanced with ethical considerations and environmental safety measures. Continued research, regulatory oversight, and public engagement are essential to maximize benefits while mitigating risks. Advancements in gene-editing technologies, such as CRISPR, may further enhance the precision and efficiency of transgenic modifications in the future.

***Keywords**:* biotechnology, biopharming, ethical concerns, genetic engineering, Transgenic animals.

1.0 INTRODUCTION

Transgenic animals, also known as genetically modified organisms (GMOs), are those animals whose genome has been altered through the introduction of foreign DNA from another species of animal. This foreign DNA, also called a transgene, is introduced into the fertilized egg or early embryo, allowing it to integrate into the chromosomes of the animal. As a result, the transgene is seen in all cells of the resulting organism and can be transmitted to subsequent generations. Similar to the technology of recombinant DNA, the GMOs have been studied for more than 30 years. This technology, a product of recombinant DNA technology (rDNA technology), has opened up new avenues in various fields such as medicine, agriculture, and research [1, 2, 3, 4, 5]. Conventional breeding, in contrast to animal biotechnology, can only occur between individuals of the same or closely related species for a variety of reasons, such as behavioral, temporal, and mechanical isolation mechanisms. Some species may take five to ten years, and occasionally even twenty, to produce a single generation, which is too much to handle in the modern world with the present and rapid increase in population experienced globally [4, 6, 7]. This addition ought to be made in a method that ensures the genes are passed on to the following generation or generations in addition to being introduced. One related technique for separating and creating the desired gene that results in the desired phenotype in the recipient animal is recombinant DNA (rDNA) technology [4, 8]. A gene construct is referred to as a transgene when it is incorporated and passed down into the recipient organism's genome, and the coding product—whether it be a protein or something else—that is created as a result is called

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a transgenic product [4]. Genetic modification of animals often aims to enhance certain traits or enable the animal to produce substances that are essential for human use, such as pharmaceuticals or genetically modified proteins. While the promise of transgenic animals is immense, offering advancements in medicine, agriculture, and environmental protection, there are also significant ethical, environmental, and health concerns associated with their creation. The objective of this review is to present some transgenic animals, methods of production, promises and peril of genetic modifications. This review discusses transgenesis in animals, explores genetic modification techniques, and examines the associated ethical considerations. The current landscape of research was assessed by analyzing the primary topics, key terms, and focal areas of interest.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Selection of database and identification of transgenic animals and their modifications

Based on quantitative analyses using transgenic animals and their modifications, various databases were integrated to locate global studies on these topics. Databases such as PubMed, Web of Science (WoS), and Scopus were chosen for their ease in navigating specific subject areas. The archives were thoroughly searched for QMRA-related research, and titles and abstracts were quickly screened based on the study's inclusion criteria. Additional references were identified through Google Scholar's "cited by" links. In Web of Science, documents on transgenic animals and their modifications were retrieved using both title- and topic-specific searches with the query: *(transgenesis in animals* OR transgenic animals*) AND modifications*) AND ethical concerns*, covering all publication years. In Scopus, similar articles were identified, while in PubMed, an advanced search combined with title and abstract fields was used with the expression *(transgenesis in animals* | transgenic animals*) AND modifications*, followed by filtering for abstracts, full texts, and journal articles. Using broad and general search terms ensured that the database extraction covered all relevant topics, including transgenic animals, genetic modifications, and ethical issues. Finally, the identified articles were saved into libraries across the three datasets. Computer-assisted duplicate elimination was applied by processing each article's title and removing any redundancies according to the established inclusion and exclusion criteria.

2.2 Methods

2.2.1 Data extraction

After removing duplicates and irrelevant studies, 10 relevant studies were ultimately selected for review. The limited sample size reflects the relatively sparse research conducted on transgenic animals. Extracted data from these studies included details such as the author, publication year, the specific transgenic animals involved, sample size, sample type (i.e., animal type), sampling site, observed activities around the sampling location, modification techniques, nucleic acid extraction methods, and detection techniques. A thorough re-evaluation of this extracted information was then performed to ensure high data quality and accuracy of the results.

2.2.2 Methods used to generate transgenic animals.

2.2.2.1 Vector-mediated gene transfer method

Cloning vector, denotes a small segment of DNA that contains foreign DNA and can replicate itself for the purpose of transferring or multiplying within an organism. Vectors enhance the likelihood of gene expression [5, 9]. Different types of vectors have been designed to accommodate DNA of varying lengths. Plasmids, Cosmids, the P1 phage, bacterial artificial chromosomes (BACs), and yeast artificial chromosomes (YACs) can each carry 20 kilobases (kb), 40 kb, 90 kb, 200 kb, and 1000 kb of DNA, respectively. Viruses are capable of efficiently introducing their genomes into cells. This realization led researchers to explore the potential of using viral genomes as foreign DNA vectors [10]. The following are various forms of viral vectors being used or investigated, retroviral vectors, Adeno-associated virus (AAV) vectors, and Adenoviral vectors [5, 11, 12].

2.2.2.2 DNA microinjection method

2.2.2.2.1 Pronuclear DNA microinjection

Microinjection method is the most common technique used to date for microinjection of genes into the pronuclei of zygotes. The pronuclear DNA microinjection technique in cows is shown in Fig. 1. Although pronuclear DNA microinjection has long been the most successful technique for creating transgenic offspring in pigs, only 1% of DNA-injected embryos produce transgenic animals, indicating that the effectiveness of transgenic offspring production is still

limited in this species [5, 13]. The pronuclear DNA microinjection approach has a poor success rate that varies depending on the species [5, 14]. Although the causes of this difference are unknown, they most likely have to do with modifications to the host genome's intrinsic DNA integration process or DNA repair mechanism. Additionally, the method utilized to make the artificial molecules (promoters and coding sections), and other cellular machinery-related characteristics, exogenous DNA purity, may be the cause of low transgenesis efficiency in domestic animals [5, 14]. This method was initially applied to sheep, pigs, and rabbits in the 1980s, and then to goats and cows. This method's applicability to domesticated animals is still restricted [15]. This method's main flaw is that some copies of the foreign gene are haphazardly incorporated into the host genome, disrupting the expression of both the transgenic and the host gene.

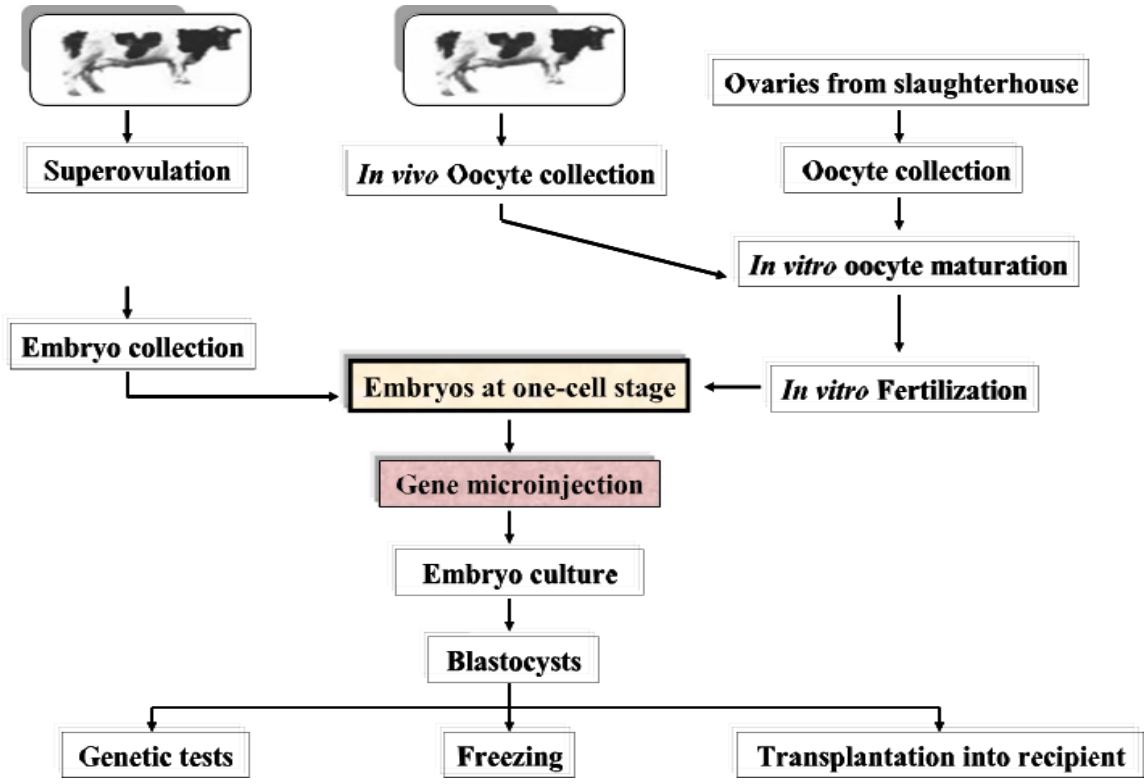

Fig. 1 Showing the pronuclear DNA microinjection technique in cow [5].

2.2.2.2.2 Embryonic Stem Cells (ESCs)

Stem cells are the cells that are present in all multicellular organisms and have the ability to self-renew to create more stem cells as well as divide, and differentiate into a wide variety of specialized cell types. Mammals have two types of stem cells: adult stem cells found in various tissues and embryonic stem (ES) cells of blastocysts [16]. For a very long time, embryonic stem cells have been developed in vitro [17]. Using homologous recombination, the appropriate DNA sequence is introduced into an in vitro culture of embryonic stem (ES) cells. It is possible to introduce foreign DNA into ES cells and create clones with the foreign gene by using a selection gene. These cells can be used to create chimeric transgenic mice (Fig. 2). The transgene in these animals is mosaic [18]. When a leukemia inhibitory factor (LIF) is added to the culture in the lab, the stem cells do not develop.

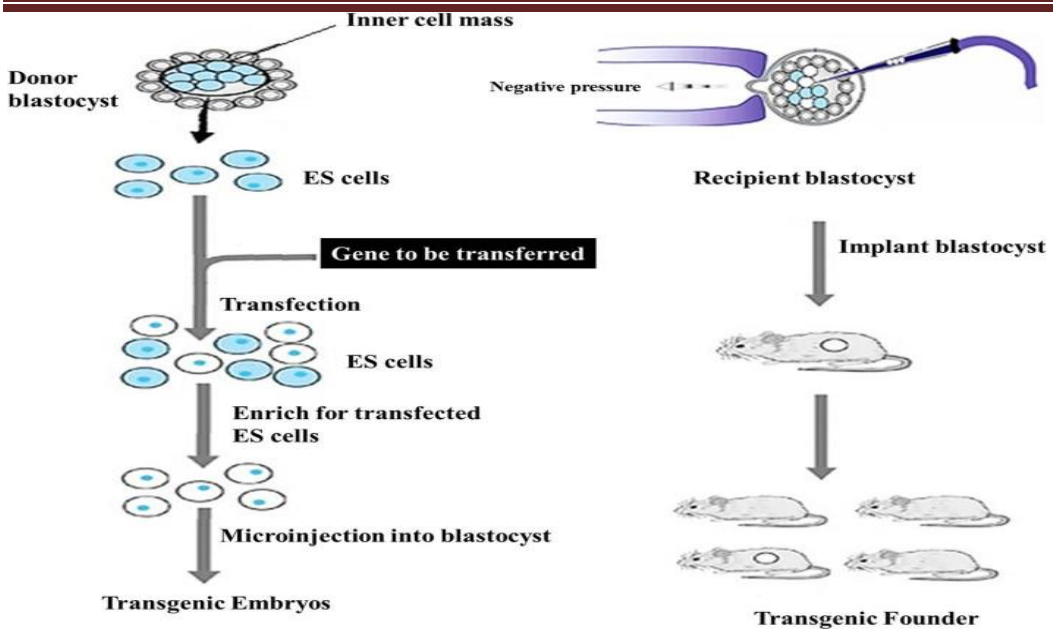

Figure 2: The DNA microinjection technique using ES cells [5].

2.2.3 Gene transfer in gametes

2.2.3.1 Sperm-mediated gene transfer technique (SMGT)

Brackett *et al*. [19] provided the first evidence that foreign DNA might be incorporated into untreated sperm. For the first time, [5, 20] showed that (a) plasmid DNA molecules can be spontaneously incorporated into mouse epididymal sperm, (b) plasmid-containing sperm cells can be used for in vitro fertilization procedures to create genetically modified offspring, (c) exogenous DNA sequences are expressed in the progenitors, and (d) sperm-carried exogenous DNA is incorporated into the fertilized ovum (Fig. 3).

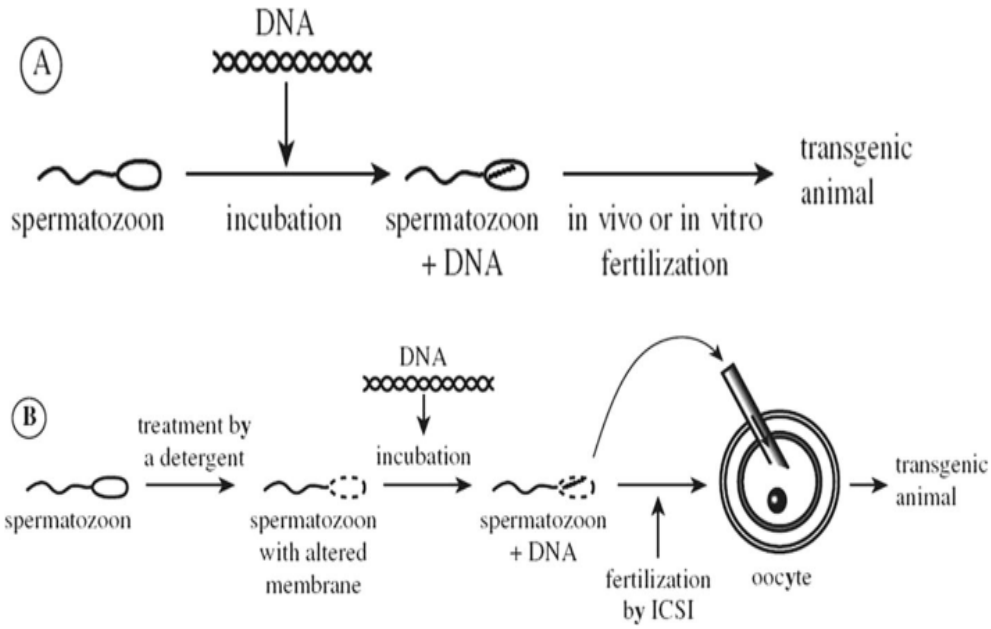

Figure 3: The sperm-mediated gene transfer technique [5].

Another intriguing aspect of using sperm as DNA vectors is the concept of mass transgenesis [21, 22]. In later studies, the successful introduction of the exogenous GH expression vector into the sperm head allowed for the production of GH-transgenic sheep characterized by a high growth rate in order to reduce the meat shortage in Egypt [22]. Transgenic mice, rabbits, pigs, sheep, cows, chickens, and fish have been created by incubating sperm cells with foreign DNA and fertilizing them in vitro or in vivo [21, 22]. Additionally, this procedure does not require any special tools or expertise and can be done in the field. According to Wu *et al*. [23], a complex structure of molecules from the class 2 major histocompatibility complex, located in the posterior region of the sperm head, mediates the primary binding site of foreign DNA in mouse sperm. Researchers found two components in the mouse seminal plasma: a variety of foreign DNA-binding proteins from the prostate and a DNase from the seminal vesicle. These components have been demonstrated to limit exogenous DNA sequestration [24, 25]. By utilizing the farmers' common artificial insemination (AI) technique, SMGT is used in domestic animals including pigs and cats [26]. Donor animals' fresh semen is washed multiple times before being centrifuged to extract seminal plasma. To enhance DNA uptake in sperm, methods such as animal artificial insemination, incubating sperm cell suspensions with foreign plasmid DNA for approximately one hour at 18, and dilution in appropriate extender dimethyl sulfoxide (DMSO) and Triton X-100, a mild polar detergent, were employed [21, 22]. As a result, the sperm membrane became unstable, giving foreign DNA complete access to the sperm. Similar results have also been obtained by freezing and thawing sperm [27]. A further intriguing alternative technique is intracytoplasmic sperm injection (ICSI), which entails inserting treated and incubated sperm directly into the oocyte. Long segments of DNA have been successfully transferred in mice, yeast, bacteria, and other artificial chromosomal constructs (YACs, BACs, and MACs) via ICSI [28, 29]. An interesting technique for creating transgenic mice is described by Chang *et al*. [30], which entails treating sperm cells with monoclonal antibodies (mAb C) and tagged foreign DNA. A simple protein called mAb C binds to DNA by ionic interactions, enabling the selective attachment of foreign DNA to sperm. This linker protein reacts with the surface antigen on the sperm of every animal under study, including pigs, mice, chickens, cows, goats, sheep, and humans. It is noteworthy that a significant component of the biology of sexually reproducing organisms is the mediating processes of foreign DNA uptake [25].

2.2.3.2 Testis-mediated gene transfer technique (TMGT)

Testis-mediated gene transfer (TMGT) (Fig. 4), is the simplest form of SMGT because testis is considered as an immune-privileged organ, and in-vitro fertilization (IVF) or embryo transfer is not needed [5, 31]. Transfer of gene in vivo into specific testicular cell types should provide an insight on how to study the molecular regulation of spermatogenesis [5, 32].

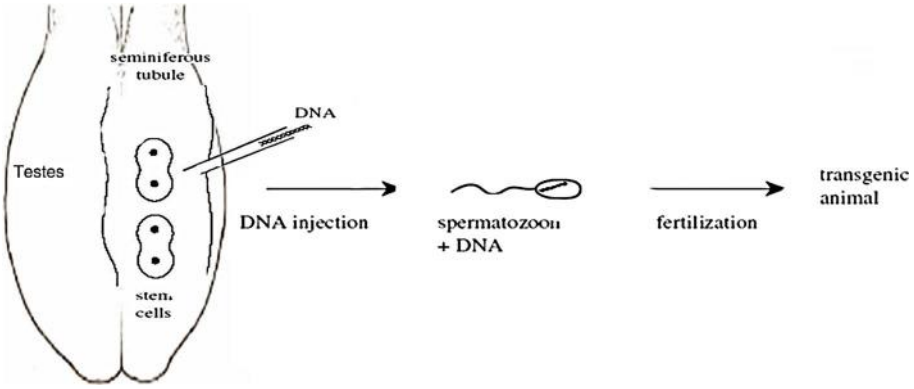

Figure 4: Testis-mediated gene transfer (TMGT) technique [5].

Insertion of foreign DNA into the testis, is considered as the fastest transferred to the epididymal ducts through the rete testis, and efferent ducts, where it is stably integrated by epididymal epithelial cells and epididymal spermatozoa [5, 33]. Another method of introducing foreign gene, is the injection of adenovirus vector solution into the interstitial space (intratesticular injection) or seminiferous tubules (intratubular injection) of the mouse testis [5, 31]. The adenovirus-mediated gene transfer may be useful for transfecting testicular somatic cells, and this method may be used for in vivo

gene therapy in the future, for treatment of male infertility, although slight impairment in spermatogenesis and inflammatory response are the major limitations associated in this method.
Xiangyang, [31] & Shakweer *et al*. [5], also suggest that production of transgenic animals and fetal gene therapy could employed testis-mediated gene transfer method.

2.2.4 Somatic cell nuclear transfer (SCNT)
Somatic cell nuclear transfer (SCNT), is a laboratory technique which involves taking the nucleus of a somatic cell (any cell other than a sperm or egg cell), and transferring it into an egg cell that has had its own nucleus removed, leading to the formation of zygote [5, 16, 34, 35]. The outcome is a reconstructed egg that can be stimulated to develop into an embryo. In mammals, the zygote needs to be artificially implanted into a surrogate mother's uterus for further development [36, 37]. Therapeutic and reproductive cloning, employed SCNT [16]. The aim of therapeutic cloning is to produce embryonic stem cells that can be used to generate tissues or organs for transplantation, while the objective of reproductive cloning, is to create a genetically identical copy of an existing animal [16]. In this view, transgenic embryos and animals are defined as those produced through nuclear transfer of genetically changed cells, as they carry the initial changes present in the nucleus of the donor cell from which the animal was derived (Fig. 5). Willadsen recorded his first significant success with SCNT in 1986, when he produced lambs cloned from embryo nuclei at stages ranging from 8 to 16 cells. This discovery triggered the interest of researchers in using nuclear transfer to multiply embryos derived from high-value agricultural animals [38]. The first mammal to be cloned using SCNT in 1996 was Dolly the sheep. Since then, many other animals (dogs, cats, goats, horses, and pigs), have been cloned using this method [16].

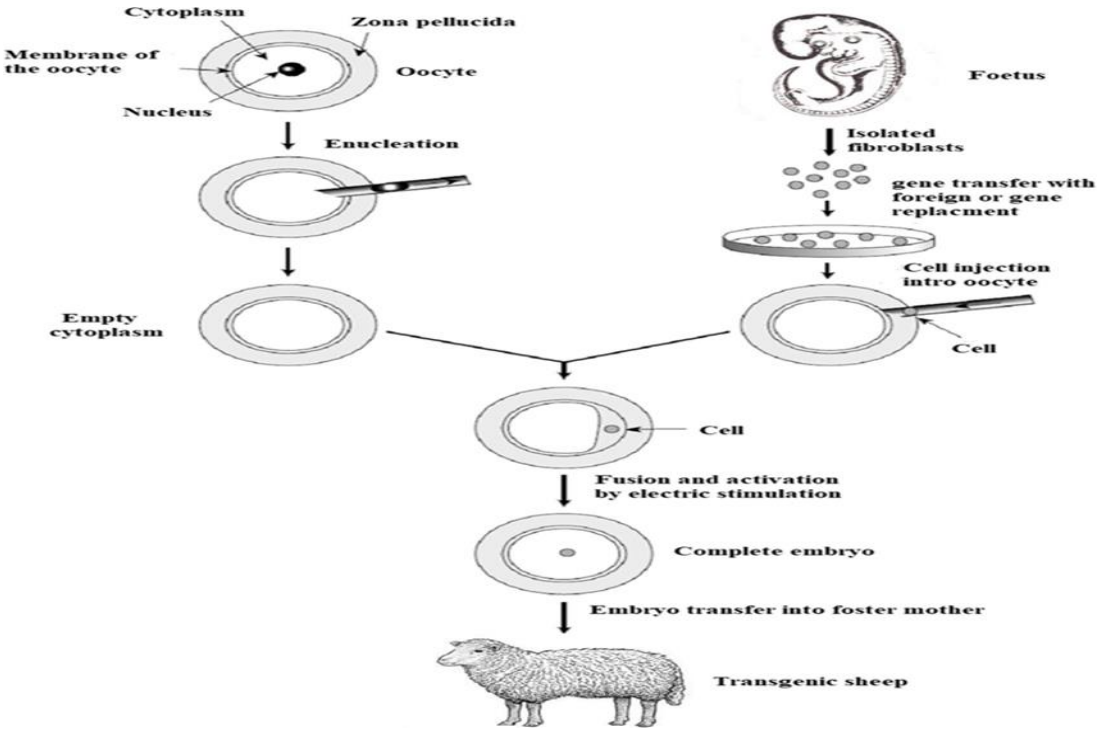

Figure 5: The somatic cell nuclear transfer technique [5].

4. DISCUSSION
4.1 Promises of genetic modifications of animals
4.1.1 As disease model

Since mice and humans share many physiological, anatomical, and genetic traits, rodents have long been used to simulate human disease. Transgenic animals, or animals genetically altered to display disease symptoms so that an effective treatment may be researched, are created as disease models for conditions like sickle cell anemia, amyotrophic lateral sclerosis, chronic hypertension, renal degeneration, osteogenesis imperf Eita, cystic fibrosis, mitochondrial cardiomyopathy, neurodegenerative disease, Werner syndrome, rhodopsin mutation, retinitis pigmentosa, melanoma, Alzheimer's disease, prostate cancer and atherosclerosis AIDS, cancer, and Alzheimer's [39]. Scientists can better understand how genes contribute to particular diseases by using transgenic animals. The potential for replacing higher species with lower species by creating disease models in mice instead of dogs or non-human primates and the degree of discomfort experienced by parent animals during experimental procedures are some advantages of using transgenic animals. Transgenic animals, like mice, have been useful for studying many genetic disorders and for investigating gene function [7, 40, 41].

4.1.2 As food

The FDA recommended that humans could safely consume cloned animals and their byproducts [7, 42]. Their muscle hypertrophy is linked to certain disadvantages, such as poor calving success that necessitates Caesareans, low calf viability, and low fertility.

4.1.3 Transgenic eggs

Eggs are a cost-effective source of high-quality protein, yet numerous individuals steer clear of them due to their high cholesterol content. Modifying the gene that governs cholesterol production could lead to the creation of healthier eggs with lower cholesterol levels. Low cholesterol eggs may help mitigate various health issues in people [43]. Recently, researchers succeeded in expressing chicken anti-Prion single-chain antibodies in transgenic quail eggs by utilizing the β-actin promoter. They also evaluated the biological activity of this protein through western blotting analysis [44].

4.1.4 Transgenic seminal vesicle

Dyck *et al*. [45], successfully expressed human growth hormone in the seminal vesicles of transgenic mice by utilizing a mouse P12 gene promoter. They achieved a concentration of 0.5 mg/ml, demonstrating a viable alternative to utilizing the mammary gland as a bioreactor. The preferred animal for this type of expression is the boar, as it can produce ejaculate volumes of 200-300 ml per ejaculation, with a total protein concentration of 30 mg/ml, and can ejaculate 2-3 times weekly, throughout the year [46]. Consequently, a single transgenic pig can express foreign proteins under the regulation of a comparable promoter specific to accessory sex gland at a rate of 1.0 mg/ml could produce 22.4gm of protein/ year.

4.1.5 Transgenic blood

Swanson *et al*. [47] were able to produce human hemoglobin in the blood of genetically modified pigs. This hemoglobin could be extracted from their circulation and utilized to develop a blood substitute for human patients, as pigs are relatively easy to breed, thus ensuring a continuous and cost-effective source of hemoglobin. This resource could potentially address the issue of blood shortages.

4.1.6 As bioreactor

The production of therapeutic proteins was one of the earliest applications of recombinant DNA technology. By 2003, the European Union had approved 88 recombinant protein-based products. However, none of these approved products had been derived from transgenic systems. Despite this, domestic animals offer an efficient system for producing large, complex, and biologically active recombinant proteins that can be used to treat or prevent human diseases. The concept of producing pharmaceutical proteins in the mammary glands of livestock led to the term *biopharming* [48] or *gene pharming* [49]. Researchers have successfully developed transgenic rabbits, sheep, goats, pigs, and cattle that express heterologous proteins for medical applications. Biopharmaceuticals produced through transgenic animals serve a wide range of purposes, including the treatment of multiple sclerosis, hepatitis, cystic fibrosis, blood disorders, certain cancers, hemophilia, thrombosis, growth disorders, Pompe's disease, osteoporosis, Paget's disease, and anemia. Additionally, these advancements have contributed to the development of improved infant formula. Initially, research on transgenic animals as bioreactors focused on using mammary glands as the primary site for protein production. However, more recent studies have explored other biological systems, including blood, the bladder, eggs, and male accessory glands, as potential bioreactors for pharmaceutical protein production.

4.1.7 Drug and Industrial production

Transgenic animals are utilized for the production of essential proteins, such as alpha-1-antitrypsin, which is produced in the liver and used to treat conditions like emphysema and cystic fibrosis. This method is more cost-effective than producing proteins through human cell cultures [50]. The human lungs are constantly exposed to foreign particles like dust, spores, and bacteria. To counter these, neutrophils release the elastase enzyme, which helps break down harmful substances. However, elastase can also degrade elastin, a crucial component that maintains lung elasticity. To prevent this damage, the human body produces α1-proteinase inhibitor, a protein that has been successfully expressed in transgenic sheep [51]. Recombinant human proteins are now commonly produced in the mammary glands of transgenic animals, marking a significant advancement in biotechnology [52, 53]. Pharmaceutical proteins derived from these methods are increasingly being used for commercial purposes [54, 55]. In a notable example, two scientists at Nexia Biotechnologies in Canada successfully spliced spider genes into the cells of lactating goats, demonstrating the potential of genetic engineering in biopharmaceutical production. Genetically modified goats are being used to produce silk proteins in their milk, which can be extracted and woven into lightweight yet durable threads. This material has potential applications in manufacturing military uniforms, medical micro-sutures, and tennis racket strings. Studies indicate that 60% of Americans support the use of transgenic animals for such purposes. Additionally, the mammary glands of transgenic goats are utilized for the production of monoclonal antibodies, while transgenic cattle have been engineered to produce recombinant bispecific antibodies in their blood, advancing biomedical research and therapeutic applications [56]. Another breakthrough in biotechnology is the development of trans-chromosomal animals. In this approach, a human artificial chromosome containing the complete sequences of the human immunoglobulin heavy and light chain loci is introduced into bovine fibroblasts, which are then used in nuclear transfer. As a result, trans-chromosomal cattle have been produced that express human immunoglobulin in their blood. This advancement represents a significant step toward the large-scale production of human therapeutic polyclonal antibodies, which could have important applications in treating various diseases [7, 57].

4.1.8 As disease control

Scientists in Australia genetically modified the mousepox virus to alter its genes [58]. Some researchers have also explored the possibility of developing genetically modified mosquitoes that are incapable of transmitting malaria. However, other scientists have raised concerns about the potential unforeseen risks these modified mosquitoes could pose if released into the environment [7, 59].

4.1.9 Xenotransplantation

Today, approximately 250,000 people are alive due to successful organ transplantation. However, the shortage of suitable donor organs and the risk of rejection remain significant challenges in allotransplantation. To address this issue, genetically modified pigs have emerged as a promising source for xenotransplantation, offering an alternative to human organ donation. Porcine xenografts from domesticated pigs are considered the best option for organ and tissue transplantation [7, 59, 60]. By deleting specific genes responsible for triggering the human immune system's rapid rejection response, genetically modified pigs can provide viable organs for human recipients. In Canada, a national survey on xenotransplantation found that only 48% of respondents considered the use of animals as a source of living cells, tissues, or organs to prolong human life acceptable. To overcome hyperacute and acute vascular rejection, transgenic pigs have been engineered to produce human complement regulatory proteins. Studies show that transplanting porcine hearts or kidneys expressing these regulators into immunosuppressed nonhuman primates has resulted in survival rates ranging from 23 to 135 days, demonstrating that hyperacute rejection can be managed in a clinically acceptable manner. For long-term graft tolerance, researchers are exploring strategies such as inducing permanent chimerism through intraportal injection of embryonic stem (ES) cells or co-transplantation of vascularized tissues. These advancements represent critical steps toward making xenotransplantation a viable and sustainable solution for addressing the global organ shortage.

4.1.10 Agriculture

Transgenic pigs carrying a human metallothionein promoter or a porcine growth hormone gene construct have demonstrated significant improvements in economically important traits, including increased growth rates and improved body fat-to-muscle ratios [7, 61, 62]. In food production, transgenic pigs have been developed using a spinach desaturase gene, which enables them to produce higher amounts of unsaturated fatty acids. This innovation not only enhances the

nutritional quality of pork but also helps reduce the risk of stroke and coronary disease [63, 64]. Similarly, transgenic animals have been used to enhance milk production, leading to improvements in milk composition. While transgenic mice have been widely studied for this purpose, some unintended side effects have also been observed [7, 65, 66]. In pigs, genetic modifications have been used to increase milk production by altering lactose composition [67]. Specifically, the transgenic expression of a bovine lactalbumin construct in sow milk has resulted in higher lactose content and increased milk yields [68]. These improvements have been linked to better survival rates and enhanced development of piglets. Transgenic sheep have also been engineered to improve wool production. Sheep carrying a keratin-IGF-I construct exhibited increased gene expression in their skin, leading to a 6.2% increase in clear fleece yield compared to non-transgenic animals [69, 70]. Beyond enhancing productivity, scientists are also working to develop disease-resistant livestock. Efforts to create influenza-resistant pigs are underway, although the number of genes currently known to confer disease resistance in farm animals remains limited. As research continues, transgenic technology has the potential to revolutionize both agricultural efficiency and animal health [7, 71].

4.1.11 Transgenic Animals in Biopharmaceutical Production and Disease Prevention

Human milk lysozyme plays a crucial role in innate immunity, but human breast milk is not a commercially viable source of this enzyme. Lysozyme is one of the most important antibacterial components in milk, as it hydrolyzes the glycosidic β-linkage between *N-acetylmuramic acid* and *N-acetylglucosamine* in the peptidoglycan layer of bacterial cell walls [72]. Notably, lysozyme in milk exhibits three times the lytic activity of egg white lysozyme due to its greater positive charge [73]. This enzyme supports the growth of beneficial gut microorganisms in infants and enhances their disease resistance. Yu *et al*. [74], successfully expressed human lysozyme in the milk of transgenic mice, demonstrating its biological activity. Expression of this enzyme in cow's milk could potentially enhance disease resistance in vulnerable populations, including orphaned children. Similarly, Limonta *et al*. [75] produced transgenic rabbits for the production of human growth hormone using a whey acid protein gene promoter. This hormone has proven beneficial in treating growth-retarded children. Additionally, recombinant pig growth hormone has been shown to significantly increase muscle mass [76] and is used to enhance body weight in pigs. Lactoferrin, an iron-binding glycoprotein present in human milk, possesses both bacteriostatic and bactericidal properties, offering protection against infections caused by gram-positive and gram-negative bacteria. This protein plays a critical role in preventing digestive infections that result in the death of millions of newborns worldwide. A transgenic bull named Herman was developed to carry the human lactoferrin gene. Produced by Gene Pharming Europe B.V. (Netherlands), Herman has successfully sired multiple offspring [77]. The respiratory system is constantly exposed to airborne microorganisms. Neutrophils in the lungs secrete elastase to eliminate these pathogens. However, elastase can also degrade elastin in the alveolar walls, reducing lung elasticity and leading to emphysema. To counteract this, the body produces α1-protease inhibitor (formerly known as α1-antitrypsin or α-PI). This enzyme has been successfully expressed in transgenic sheep, with a notable example being *Tracy*, a sheep developed by PPL Therapeutics (UK). The purified protein is now used for treating lung emphysema [77]. Several biotechnology companies are pioneering the production of therapeutic proteins in transgenic animals. GTC Biotherapeutics has developed a *C1 inhibitor* in transgenic rabbit milk for the treatment of hereditary angioedema. Similarly, Pharming Group N.V. has explored recombinant protein production in various livestock species. One remarkable innovation is *Bio-Steel*, a high-strength fiber derived from spider silk proteins. Among the strongest natural fibers on Earth, spider silk has potential applications in bulletproof vests and surgical sutures. Nexia Biotechnologies (Canada) successfully transferred spider silk genes into goats, enabling the production of spider silk proteins in goat milk [43]. In 1997, *Polly*, a transgenic sheep, was developed to express human blood clotting factor IX in its milk. This was achieved using nuclear transfer technology. Factor IX plays a crucial role in blood coagulation and is essential for treating hemophilia B. Additionally, human antithrombin III (AT-III), a natural anticoagulant present in human blood, is vital for preventing abnormal clot formation. Individuals with AT-III deficiency are at high risk of developing life-threatening blood clots, which can lodge in the lungs or brain. To address this, Genzyme Corporation successfully developed AT-III-producing transgenic goats, providing a reliable source of this therapeutic protein. A variety of human proteins have been successfully expressed in the milk of transgenic animals, including *insulin-like growth factor I* in rabbits, *α-lactalbumin* in cows, and *protein C* in pigs [53]. Furthermore, *α-glucosidase* extracted from transgenic rabbit milk has been used to treat Pompe's disease in infants [78]. Among farm animals, rabbits are the preferred species for transgenic protein production due to their early maturity, short gestation period, and high reproductive rates compared to larger livestock species. As research advances, transgenic animals continue to play a vital role in biopharmaceutical production, offering novel solutions for treating human diseases and enhancing global healthcare.

4.2 Perils of genetic modifications of animals

One of the primary environmental concerns associated with transgenic animals is the risk of their escape into the wild. The potential consequences vary depending on the species and the transgene involved. While many farm animals are confined and unlikely to survive in the wild, certain transgenic species such as fish, pose greater ecological risks. Transgenic animals are predominantly utilized for studying gene functions, modeling human and animal diseases, and testing experimental pharmaceuticals [79]. They also hold promise as a potential source of human organs for transplantation. However, despite their significant contributions to agriculture and biomedicine, the use of transgenic animals presents both ethical and environmental challenges. Scientists must actively engage in discussions regarding the ethical implications of this technology, its potential effects on ecosystems, and its impact on farmers and consumers. The development and implementation of transgenic technologies are neither simple, cost-effective, nor highly efficient, necessitating careful evaluation of their risks and benefits [80]. To address the ecological concerns associated with transgenic organisms, the field of "biosafety" has recently emerged [79]. This area of research aims to assess and mitigate the potential environmental impacts of transgenic animals. Due to the high costs involved in generating transgenic organisms, only a limited number of animals can be produced. Consequently, maintaining genetic diversity through backcrossing becomes a complex process. To improve economic feasibility, advances in assisted reproductive technologies, such as artificial insemination, embryo transfer, and in vitro embryo production must be leveraged [3, 6]. The primary ethical concern regarding transgenic animals is their use in studying human diseases. Similar concerns apply when animals are genetically modified to produce pharmaceutical proteins or serve as organ donors, as embryo manipulation can negatively impact animal welfare [81, 82]. Regulatory commissions, experienced in evaluating the risks of conventional pharmaceuticals, are now assessing the medical implications of using transgenic animals for therapeutic protein production [83, 84]. While transgenic animals are generally considered to pose minimal environmental risks, specific concerns arise in cases such as transgenic fish and live-virus-based vaccines, which require complex environmental risk assessments [85]. Research by Muir & Howard [86], suggests that growth hormone (GH)-transgenic fish exhibit rapid growth and early sexual maturity but tend to have shorter lifespans and increased fragility compared to non-transgenic counterparts. If released into the wild, such genetically modified fish could contribute to the local extinction of native species. Although this scenario remains unlikely, it cannot be completely dismissed. As a result, regulatory bodies have yet to approve the commercial breeding of fast-growing transgenic fish. Potential solutions to mitigate these risks include sterilizing female transgenic fish or restricting fish farms to closed environments to prevent unintended releases. Regulatory agencies may permit the consumption of genetically modified fish while prohibiting their reproduction. Assessing the impact of transgenic animals on biodiversity requires ongoing research and regular updates. Governments and scientific institutions must continuously monitor and evaluate the ecological implications of emerging transgenic species to ensure responsible and sustainable advancements in this field [86].

5.0 CONCLUSION

Genetic engineering enables the incorporation of foreign genes into animal genomes, allowing these modifications to be inherited and expressed by subsequent generations. Through genetic modifications of animals, scientists can address current and future human needs in agriculture, food production, and resource management. By developing disease-resistant animals and enhancing livestock productivity, transgenesis has the potential to reduce reliance on traditional pharmaceuticals and improve global food security. Beyond agriculture, transgenic animals play a crucial role in advancing human health. They offer potential solutions to organ shortages and serve as bioreactors for producing essential pharmaceuticals used in treating various human diseases. However, the perils and animal welfare considerations present significant challenges to the widespread adoption of these technologies. To facilitate responsible implementation, it is essential to enhance the efficiency of transgenesis and increase public awareness to mitigate opposition to this emerging field.

Acknowledgment

We acknowledged Mr. Emmanuel Nyoho, for assisted us by providing Internet facilities for the success of this review article.

Authors contributions

Owoidihe M. Etukudo conceptualized the work, and wrote the manuscript, while Nko S. Bassey sourced for material from internet and assembled them for the work. All authors read and approved the manuscript.

Conflict of interest
The authors declared no conflict of interests.

Funding
The authors declared no source of external funding.

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