Scientific Works. Series C. Veterinary Medicine. Vol. LXXI (1), 2025
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

# TRANSGENIC ANIMALS: INNOVATIONS, APPLICATIONS, AND ETHICAL CONSIDERATIONS IN MODERN BIOTECHNOLOGY

**Raluca-Aniela GHEORGHE-IRIMIA[1], Dana TĂPĂLOAGĂ[1*], Cosmin ȘONEA[1], Lucian-Ionel ILIE[1], Makki Khalaf Hussein AL DULAIMI[3], Alexandru USTUROI[4], Paul-Rodian TĂPĂLOAGĂ[2]**

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, 5th District, 050097, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Productions Engineering and Management, 59 Mărăști Boulevard, 1st District, 011464, Bucharest, Romania
[3]Al-Furat Al-Awsat Technical University, Baghdad, Iraq
[4]"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iași, Romania

*Corresponding author email: drtapaloaga@yahoo.com

***Abstract***

*Transgenic animals represent a key achievement in modern biotechnology, providing novel solutions in industries such as agriculture, medicine, and environmental studies. Techniques such as microinjection, somatic cell nuclear transfer, and CRISPR/Cas9 gene editing have facilitated the development of transgenic animals by allowing precise genetic modifications. These advancements have led to the development of livestock that not only exhibit improved growth rates and disease resistance but also serve as bioreactors to produce human proteins and monoclonal antibodies. However, the application of transgenic technology raises significant ethical considerations, particularly regarding animal welfare and ecological impacts. The potential for unintended consequences, such as insertional mutagenesis and epigenetic silencing of transgenes, needs thorough evaluation of the long-term effects on both the transgenic animals and their ecosystems. Furthermore, public perception and regulatory frameworks around genetic engineering must be addressed to ensure responsible development and application of these technologies. This mini-review aims to synthesise current advances in transgenic animal technology, investigate their various applications, and critically evaluate the ethical concerns of their use.*

***Key words**: transgenic animals, biotechnology, genetic engineering.*

## INTRODUCTION

In the present day, molecular biology is undergoing a remarkable pace of advancement. One of them is the capacity to create transgenic animals. A transgenic animal is an organism whose genome has been altered to incorporate genes from another species or to utilise techniques for animal genome editing to achieve particular traits. The gene (or genes) can be intentionally modified to modify the appearance of an animal (Emmanuel et al., 2018).

In this regard, transpharmers are transgenic animals that are employed in pharmaceutical production. They generate critical human proteins in their milk and eggs as a result of the insertion of human genes. ATIII, α-antitrypsin, and tPA are among the products that have been produced from sheep milk thus far (Gheorghe-Irimia, 2023). Additionally, blood, seminal plasma, urine, silk gland, and insect larvae haemolymph are theoretically viable systems (Wang et al., 2013; Maksimenko et al., 2014; Шепелев et al., 2018). Comparably, transgenic mice have become indispensable models for investigating human disorders since they enable exact gene editing and monitoring of consequent phenotypes (Christapher et al., 2022).

Moreover, transgenesis enables the enhancement of the quality of the entire diet, the quantity of nutrients, and the specific nutritional composition of animal products. Transgenic technology has the potential to facilitate the transfer or enhancement of nutritionally advantageous characteristics (Gheorghe-Irimia, 2024; Șonea et al, 2023a; Șonea et al., 2023b).

Notable techniques include microinjection, which has been routinely employed since the introduction of transgenic technology and directly delivers DNA into the pronucleus of fertilised eggs (Nagano et al., 2001). Additionally, the incorporation of transformed somatic cells into recipient oocytes is facilitated by procedures like somatic cell nuclear transfer (SCNT), which in turn facilitates the production of transgenic progeny. Recent advancements have introduced targeted genome editing tools, including CRISPR/Cas9, which enables precise modifications at predetermined genomic loci, thereby enhancing the precision of transgene integration and minimising off-target effects (Park et al., 2016). Lentiviral vectors are also effective tools for introducing genes into a variety of animal species, which leads to a high level of transgenic efficiency, particularly in porcine and bovine models (Hofmann et al., 2003). In addition, sperm-mediated gene transfer (SMGT) offers an additional approach to the development of transgenic animals by incorporating genetic material into sperm, which is subsequently utilised for *in vitro* fertilisation (Lavitrano et al., 2002). These developments emphasise the expansion of methods for the development of transgenic animals, which is fostering significant applications in biomedicine and research.

This review aims to investigate the most current advancements in transgenic animal technology, investigate their several applications in biotechnology, and assess their ethical and legal conundrums in use.

## TECHNIQUES USED FOR TRANSGENIC ANIMALS' GENERATION

Over the past few decades, a variety of techniques have been employed to obtain transgenic animals. Numerous sequences have been identified as the result of gene sequencing, which has contributed to the understanding of genes and promoters that are relevant to numerous species. The emergence of genomics, proteomics, and a new generation of reproductive biotechnologies all suggest that successful transgenic applications in domestic animals are imminent. The intended purpose of a transgenic animal dictates the procedures and methodologies employed in its development (Wang et al., 2016).

Microinjection is a key technique for generating transgenic animals that involves directly inserting DNA into the pronucleus of fertilised oocytes. This method allows the precise delivery of genetic material, which could contain single or multiple genes meant for expression in the resulting organism. The method begins with the isolation of one-cell fertilised embryos then injects a DNA solution into the pronucleus, which has genetic material derived from sperm (Moreira et al., 2016). The microinjected DNA can integrate into the host genome, but with varying effectiveness; historical evidence indicates that roughly 5% of injection embryos effectively incorporate the transgene (Cho et al., 2009). The intrinsic benefits of microinjection include its simplicity and versatility, which allow for the introduction of numerous types of nucleic acids such as RNA or proteins, increasing its uses beyond DNA (Wudarski et al., 2017). Notably, the effectiveness of transgenic integration frequently depends on cellular repair processes that handle the injected material, resulting in either stable integration or temporary expression (Smirnov et al., 2019). Recent research shows that microinjection continues to support advances in transgenic technologies, such as the use of CRISPR for genome editing, which improves the accuracy of genetic alterations (Zabelina et al., 2022; Harms et al., 2014).

In animal cloning, somatic cell nuclear transfer (SCNT) is the process of converting the genetic material of a somatic cell into an enucleated egg therefore generating a viable embryo. Following the successful cloning of Dolly the sheep in 1996 - a major turning point in reproductive biotechnology - this approach became well-known (Simmet et al., 2020; Ogura et al., 2013). SCNT has applications in genetic modification, particularly in transgenic animals' generation, because it allows researchers to use genetically modified somatic cells as the nuclear donor. This allows for the targeted modification or improvement of traits in the subsequent offspring (Matoba et al., 2014; Bordignon et al., 2013). According to research, SCNT can be used to create clones of genetically enhanced livestock, conserving and transmitting desired phenotypic features while also contributing in species conservation. Moreover, strategies for maximising reprogramming during SCNT, including raising histone demethylation, have been looked at to raise embryo survival and development (Eun et al., 2017; Simmet et al., 2020).

Another major advancement in the field of genetic engineering is CRISPR/Cas9 gene editing, which offers more accuracy in genome editing. Using a guide RNA (gRNA) component, the method allows the Cas9 nuclease find a particular DNA sequence, thereby enabling site-specific modifications including gene knockouts, insertions, or substitutions (Wang et al., 2016).

When compared to conventional techniques like randomised integration in transgenesis, which is usually linked with off-target effects and unpredictable results, this customised approach boosts the efficacy of gene editing (Carey et al., 2019). Creating transgenic animals by means of CRISPR/Cas9 has several benefits. Particularly in livestock, it provides shorter production cycles and reduced costs; effective gene modifications linked to muscle development and disease resistance in pigs (Whitworth et al., 2016). Furthermore, the ability to co-inject numerous gRNAs at the same time enables multiplex gene editing, allowing precise alterations at multiple loci, increasing its adaptability in both research and agriculture (Wang et al., 2015; Wang et al., 2018). Furthermore, advances in CRISPR/Cas9 technology aim to reduce off-target effects while enhancing specificity and safety in gene-edited species, which are crucial for regulatory approval and public acceptability (Han et al., 2020; Lu et al., 2023).

## APPLICATIONS OF TRANSGENIC ANIMALS

Particularly for increasing production efficiency, disease resistance, and the generation of valuable biopharmaceuticals, transgenic animals are becoming ever more significant in agriculture. Some of the practical applications of transgenesis in animal production include increased disease resistance, improved carcass composition, improved milk production and/or compositions, improved feed utilisation and growth rate, and greater prolificacy and reproductive performance. One of the most critical candidate genes utilised in the production of transgenic farm animals to enhance their milk production and growth rate is growth hormone. Another important use is the employment of transgenic animals as bioreactors for the synthesis of recombinant proteins like human butyrylcholinesterase, which can protect against organophosphate poisoning (Huang et al., 2007). Furthermore, transgenic goats can express human lysozyme in their milk, reducing susceptibility to mastitis and increasing overall productivity. Further developments include the development of disease-resistant transgenic pigs, which might greatly reduce reliance on antibiotics while improving animal welfare (Chen et al., 2019). This is aligned with sustainable agriculture principles, as limiting antibiotic use addresses growing concerns about antibiotic resistance among cattle. Furthermore, the regulatory landscape is changing as scientists work to bring transgenic inventions to market and gain public acceptance of genetically altered products (Jagadeesan and Salem, 2015; Murray & Maga, 2016).

Another important fields in transgenic animals production are biomedical research and medicine. Mice, pigs, and rabbits have been genetically engineered to mimic human diseases, allowing for extensive research into pathology and therapeutic efficacy. Because of their anatomical and physiological similarities to humans, transgenic pigs have been used as models for a variety of disorders, including cardiovascular disease, diabetes, and cystic fibrosis (Perleberg et al., 2018). This shared characteristic improves the predictive validity of medication responses and safety assessments in preclinical trials. Furthermore, transgenic rabbits are used in antibody production and are important in pharmaceutical research because they can create specific antibodies against human targets (Flisikowska et al., 2011). The use of transgenic models has also increased since the development of CRISPR/Cas9 gene editing (Tanihara et al., 2021). These developments allow researchers to create models that precisely mimic human disease processes, which is essential for testing new therapy options (Nagaya et al., 2021). Furthermore, as it was presented before, transgenic animals work as bioreactors, producing complex biopharmaceuticals such as proteins and antibodies at scalable levels (Hryhorowicz et al., 2020).

Moreover, transgenic animals have a wide range of uses in environmental management and research, notably the use of environmental DNA (eDNA) approaches. eDNA is genetic material extracted from environmental samples such as soil, water, or air, which can provide information about biodiversity and ecological health. For example, Xu et al. (2021) highlight how transgenes from genetically modified animals can be detected non-invasively using eDNA techniques, thereby assisting with wildlife monitoring and conservation activities. This feature enables researchers to evaluate the dispersion and ecological impact of transgenic animals without requiring direct observation or capture (Xu et al., 2021). Sundström et al. (2016) show that gene-environment interactions can affect eating and anti-predatory behaviours in both wild and transgenic coho salmon, emphasising the possible ecological effects of releasing transgenic organisms into the wild. Furthermore, the development of transgenic animals raises worries about their escape or release into natural habitats.

## BIOSAFETY CONSIDERATION, ETHICS AND CHALLENGES

The ethical and biosafety issues surrounding transgenic animals are complex and important in controlling their development and use. One major issue is the risk of unexpected ecological consequences, as transgenic organisms may interact unpredictably with native species and ecosystems if they escape or are deliberately released. This emphasises the significance of robust biosafety evaluations in properly evaluating risks, particularly gene flow between transgenic and non-transgenic populations (Hoenicka & Fladung, 2006; Heerwaarden et al., 2012). Previous experiences with genetically modified crops have highlighted the difficulties of limiting transgene spread in agricultural contexts, demanding strict control methods (Heerwaarden et al., 2012). Ethically, the welfare of transgenic animals must be prioritised, as genetic changes can cause unexpected health problems (Croney and Millman, 2007). Legislation governing animal welfare is changing; yet, there is frequently a gap between intended ethical norms and actual restrictions, which may prioritise economic or agricultural outcomes over animal well-being (Hedman et al., 2014). Furthermore, novel tools, such as non-invasive monitoring techniques, are being developed to improve animal welfare assessments in transgenic models, perhaps alleviating certain ethical issues (Balafas et al., 2019). From a biosafety standpoint, it is critical that the procedures and technologies used to make transgenic animals undergo extensive risk assessments, taking into account factors such as the possibility of insertional mutagenesis and long-term health consequences. Stringent biosafety regulations, aligned with international protocols such as the Cartagena Protocol on Biosafety, are required to manage the release of genetically modified organisms into the environment while also ensuring the safety of human health and biodiversity (Bannantine et al., 2018).

Regarding challenges, only a limited number of animals can be obtained as a result of the transgenic process's expensive cost. This is the reason why backcrossing is a challenging procedure that must be employed to reintroduce these animals into the existing herd. In order to render this process economically viable, it is imperative to have a comprehensive understanding of the most recent developments in assisted reproduction techniques, including in vitro embryo formation or artificial insemination and embryo transfer. Moreover, the primary environmental concern associated with transgenic animals is the potential for their escape. The risks are substantially different depending on the species and the transgene.

## CONCLUSIONS

In conclusion, transgenic animals have considerable potential for scientific research, agriculture, and biomedical applications. Researchers aim to create more exact and efficient transgenic models that precisely reflect real disease and support new treatments as genetic engineering tools - especially CRISPR/Cas9 - increasingly develop. Still, ethical and biosafety issues have to be given the greatest attention if we are to satisfy public concerns about gene flow in wild populations, environmental effects, and animal welfare.

Maintaining transparency and supporting public involvement will be vital as legal systems evolve to guarantee acceptability and cooperation in the application of transgenic technology.

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