Exploring versatile medical and allied applications of genetically modified animal milk

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ABSTRACT

The use of genetic engineering has transformed medicinal protein manufacturing in transgenic animal milk, providing a more sustainable alternative to existing systems. This analysis focuses on important breakthroughs that show the usefulness and adaptability of genetically engineered animal milk. Transgenic goat milk contains recombinant human β-defensin 3, which has significant antibacterial action. Human proinsulin, which is required for diabetes control, is successfully synthesized in transgenic cattle milk. A-1-antitrypsin is produced at high quantities in transgenic mice, ensuring functioning and efficacy. Zinc finger nucleases were utilized to develop DNA-free β-lactoglobulin knockout cows, which produced hypoallergenic milk. The production of recombinant human lactoferrin and functional human lysozyme at a large scale has been achieved using marker-free transgenic cloned cows, showcasing scalable biotechnological methods. Additionally, transgenic calves with an elevated gene dosage for beta and kappa casein demonstrate improved quality and quantity of milk. Transgenic pigs generate functional human factors VIII and IX in their milk, which are necessary for haemophilia treatment. The effective production of recombinant human serum albumin in transgenic mice, coupled with the synthesis of human tissue plasminogen activator in their milk, underscores the substantial therapeutic promise of these advancements in biotechnology. Sophisticated procedures such as somatic cell nuclear transfer, zinc finger nucleases, and marker-free cloning ensure that human genes are properly integrated and expressed in animal genomes. These improvements not only result in high yields of therapeutic proteins but also pave the path for more personalized and efficient medical treatments.

Keywords:

Genetic engineering, transgenic animal milk, recombinant protein, hypoallergenic milk, biopharmaceuticals, medical applications.

INTRODUCTION

Recently, there has been considerable attention focused on leveraging milk as a medium or conduit for delivering therapeutic proteins and medications. Genetically modified milk from transgenic cows offers a novel platform for biopharmaceutical production and administration. This review examines the advancements in using genetically altered milk as a medicine carrier, focusing on diverse research projects that illustrate its potential and applications.There are several lactation animal models used here. Goats, Pigs, Mice, Cows, and Rabbits are utilised to produce biotechnological goods. A pie chart depicts the milk-producing animal model (Fig:1).

Mastitis is characterized by the swelling of the mammary gland due to a microbial invasion [1]. It is a prevalent condition affecting dairy cattle globally [2]. Traditional antibiotic treatments produced antibiotic-resistant bacterial strains[3]. (GE) technology has been suggested as an alternate approach[4].The antibodies, termed as abzymes, represent a novel enzyme resource [5]. They have the potential to shield individuals from respiratory syncytial virus infections [6]. Damage to beta cells within the pancreatic islets leads to reduced insulin synthesis, causing elevated glucose levels in the blood and urine [7]. Type 1 diabetes is associated with significant morbidity and mortality [8]. The rapid initiation of the coagulation cascade and infiltration of innate immune cells contribute to the destruction of islets and their failure to Integrate [9,10]. ATryn® is produced using genetically modified goats to generate human antithrombin [11].Hereditary angioedema (HAE) is an uncommon condition characterised by recurring swelling episodes [12].Humans with genetic deficits of a1-antitrypsin (a1AT) are more likely to develop emphysema[13].Genomic analysis confirmed that the animal showed no unintended effects, and these genetic alterations were consistently transmitted to progeny through MOET [14]. Human lactoferrin (hLF) a glycoprotein, is essential for aiding the absorption of iron mineral in the intestines [15,16].We developed a straightforward and safe nucleofection technique [17]. Recombinant human lactoferrin (rhLF) and lysozyme are frequently used in food, pharmaceuticals, and medical treatments [18,19]. Over mammalian evolution, the composition of milk has adapted to promote the development and well-being of newborns, including the enhancement of engineered proteins [20].

Dairy cows engineered to express extra beta and kappa casein gene copies within their lactating glands produced the transgenic caseins in their milk upon stimulation with synthetic hormones [21]. Haemophilia B ranks as the second most common type of haemophilia, characterized by a deficiency in factor IX. Currently, recombinant human factor IX is manufactured using transgenic Chinese hamster ovary cells [22]. The dual-transgenic pigs were engineered to produce both recombinant human factor IX (rhFIX) and porcine lactoferrin in their milk, utilizing the bovine alpha-lactalbumin promoter for gene expression [23]. Haemophilia A is a genetic disorder. It is caused by a deficit or abnormalities in (FVIII) [24].The most prevalent protein in plasma is HAS.rHSA is an alternate source of pathogen-free HAS for therapeutic purposes [25]. The genes introduced into mouse embryos can integrate into the germ line and exhibit expression patterns resembling their natural counterparts [26,27]. WAP is detected in substantial amounts in the milk of mice [28, 29]. The findings demonstrate that the WAP gene effectively controls gene expression specifically within the mammary glands during lactation [30].

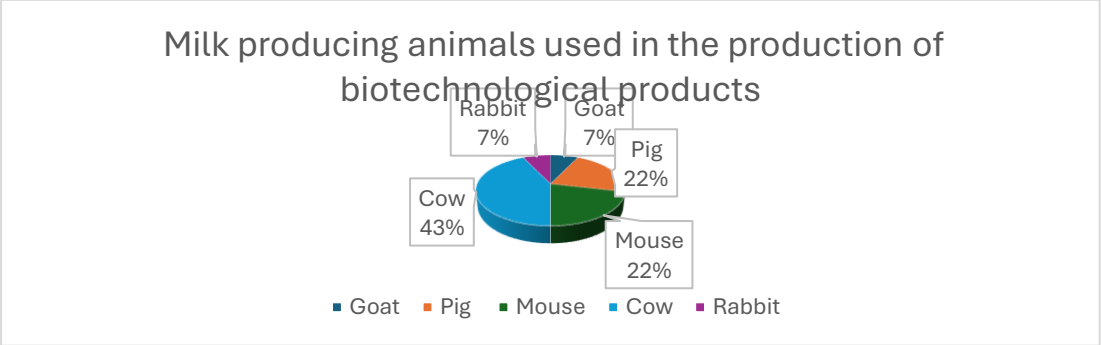

Fig:1 Milk producing animals used in the production of transgenic milk

METHODS FOR TRANSGENIC ANIMAL MILK

There are several ways used to obtain transgenic milk from milk-producing animals (Fig:2), and the results were determined based on transgenic milk analysis (Table: 1).

The process of Somatic cell nuclear transfer: Genetically modified cells were cultured in a 48-well plate. The egg underwent enucleation by removing the polar body and metaphase plate, followed by injection of a single donor cell. Electrofusion techniques were used to merge the nucleus of the donor cell with the egg.The resulting

fused embryos were then stimulated using ionomycin and 6-dimethylaminopurine, and incubated in a specific culture medium under controlled conditions of temperature and CO2 concentration [33].

Microinjection: This approach entails inserting a single artificial human chromosome, which includes the complete heavy and lambda chain loci, into bovine DNA through the use of microcell-mediated chromosome transfer and cloning methods. As a consequence of these procedures, mature and active human immunoglobulins were detected in the blood circulation of the transchromosomic calf [34].

The method of Somatic cell nuclear transfer (SCNT): Initially, 10 embryos were transferred to synchronized recipients using non-surgical methods, and pregnancy status was assessed via transrectal ultrasound on day 60. Genomic DNA analysis verified the presence of the transgene in the transgenic calf. Fibroblasts isolated from this calf were cultured in a medium containing 8 μg/mL of blasticidin to assess their antibiotic resistance [35].

Pancreatectomy, Pancreatic tissue isolation, Graft: The pancreas was enlarged using an injection of 250 mg of collagenase and neutral protease through the pancreatic duct using an 18-gauge catheter [31]. Blood samples were obtained by inserting a catheter into the jugular vein, which was flushed with saline solution after each collection. The processed pancreatic tissue was then placed into a 60-ml syringe and infused with either antithrombin or heparin at a rate of 2 ml/min. For cell injection, anesthetized mice received the cells through a catheter inserted directly into the portal vein [36].

Gene insertion: Transgenic rabbits are used to manufacture a recombinant version of human C1 esterase inhibitor (C1-INH). This biotechnological strategy involves introducing the human C1-INH gene into the rabbit genome. These genetically modified rabbits produce human C1-INH protein in their milk, which is then refined and processed into the therapeutic product used to treat hereditary angioedema (HAE) attacks [37].

Construction of hybrid gene: A chimeric gene was created by linking the Pvu II restriction site within the 5' untranslated region of the ovine BLG clone SS1 with the Taq I site located in the 5' untranslated region of a1AT. Following confirmation through DNA sequencing, the first intron of a1AT was excised. Subsequently, the P8alppg cDNA clone, which encodes the M1 variant of a1AT from its initial 80 base pairs to the BamHI site in the second exon, was inserted into the pPOLYIII-I vector. Gel-purified insert DNA from this construct was subsequently introduced into pronuclear mouse eggs to generate transgenic mice [38].

Zinc finger nuclease mRNA: ZFNs targeted a 40-base pair sequence next to a cleavage site in Exon 1 of the BLG gene, resulting in bi-allelic mutations in BLG without any residual DNA. Clone #112 exhibited bi-allelic mutations, including -17 and -16 base pair indels that caused frame-shift mutations. This clone was used as the nuclear donor for somatic cell cloning, leading to the creation of two new clones. One clone succumbed shortly after birth; however, sequencing confirmed that the surviving animal carries a bi-allelic mutation and remains in good health [39].

Somatic cell nuclear transfer process: Genetically modified cell nuclei were transferred into oocytes that had been enucleated to create reconstructed embryos in vitro, employing Electro Cell Manipulation technology 2001. Blastocysts were collected on Day 7 for subsequent implantation. A total of 492 blastocysts were implanted into 328 Chinese Luxi yellow cows, with each cow receiving 1-2 transgenic cloned blastocysts. Pregnancy was verified through ultrasonography [40].

Marker free vector method: The plasmid 706-Cre, carrying the gene for Cre recombinase, was introduced into competent DH10β cells alongside the pBAC-hLF-hLZ-Neo plasmid, which includes a floxed selection marker.. After the transformation process, each tube was placed in a shaking incubator at 30°C for 1.5 hours with 1 mL of lysogeny broth medium.The transformed cells were plated on LB agar containing 50 μg/mL kanamycin and 5 μg/mL tetracycline, followed by incubation at 30°C for 24 hours. A single colony was picked, cultured in 1 mL of LB medium at 30°C for 3 hours, and then grown overnight at 37°C. Plasmid DNA was extracted and a portion was re-transformed into the cells [41].

Somatic cell nuclear transfer method(SCNT): Constructs with extra bovine beta and kappa casein gene sequences are created and then inserted into bovine fibroblasts.The nuclei of genetically modified fibroblasts are transferred to enucleated oocytes.The resultant embryos are cultivated in vitro before being put into surrogate cows, where they grow into transgenic calves [42].

Somatic cells nuclear transfer technique: This technique was used to produce pigs with two genetic modifications, introducing the human factor IX and pig lactoferrin genes, regulated by the cow alpha-lactalbumin promoter. Oxytocin was given via intravenous injection into the sow's ear vein using a winged infusion set, starting on day 7 and continuing until day 30 postpartum to initiate lactation. While collecting milk, each sow nursed a group of 8-10 piglets concurrently [43].

Microinjection method: The full sequence of human FVIII cDNA was placed downstream of a 2.5-kb promoter from the mouse WAP gene to specifically direct gene expression to the mammary gland. This hybrid WAP-human FVIII gene construct was inserted into pig embryos and then placed into three surrogate sows. Two of these sows successfully carried the embryos to full term, resulting in the birth of around 15 piglets [44].

Expression vector method: Plasmid 807 includes elements such as a double chicken beta-globin insulator, a promoter from bovine beta-casein, and sequences near the 3' end of alpha-s1-casein, all combined using the In-Fusion HD Cloning System [45].

Microinjection technique: The WAP-tPA construct underwent enzymatic digestion with Hind-3 and BamHI, followed by fragment separation through gel electrophoresis. The 4.9 kb segment was isolated using glass fiber filter sheets, then eluted and concentrated through ethanol precipitation. The DNA solution was subsequently suspended, the solution was prepared with 10 mM Tris, 0.05 mM EDTA at pH 7.5, and had a concentration of 5 ng/µL. The genetic coding region of PWAP-tPA was microinjected into single-cell fertilized embryos [32].Tail sections of mice born after these injections were taken at four weeks of age [46].

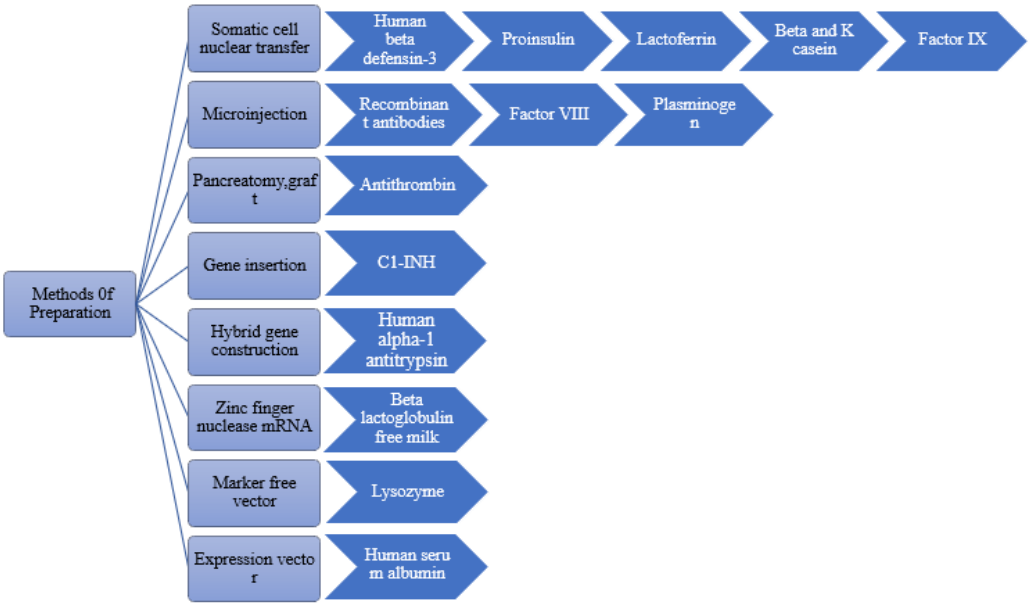

Fig:2 Classification of methods of preparing biotechnological products

CLASSIFICATION OF GENETICALLY ENGINEERED MILK PRODUCTS

This classification is based on the many types of biotechnological products derived from milk-producing animals (Fig:3), and these products are classified accordingly.

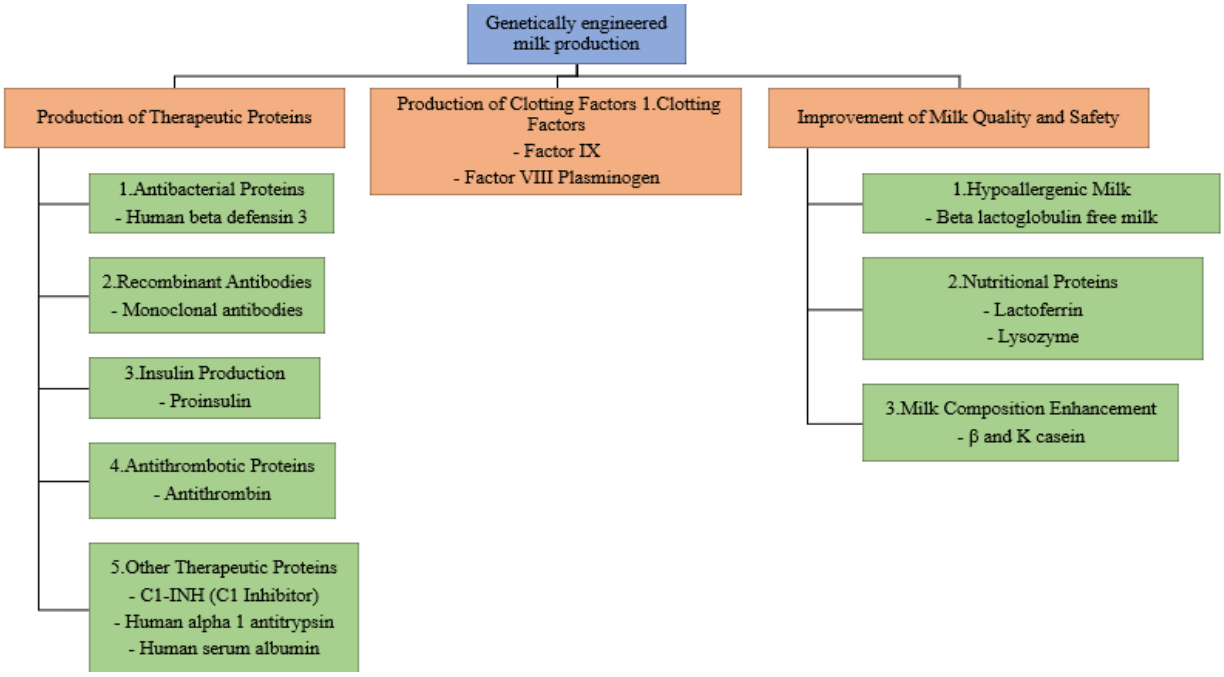

Fig:3 Classification of biotechnological products based on the different product categories obtained.

APPLICATIONS OF LACTATING TRANSGENIC ANIMAL MILK IN MEDICAL AND ALLIED FIELDS

Table:1 Applications of genetically engineered milk-producing animals in medicine and allied sectors.

Sr.no.	Transgenic animal milk product	Animal model used	Results	Reference
1.	HBD-3	Goat	HBD-3. Goat Significant antibacterial activity was observed against a variety of infections.	[33]
2.	Recombinant antibodies	Cow	High-affinity antibodies are generated effectively and at a rate comparable to established techniques	[34]
3.	Proinsulin	Cow	Successful synthesis of human proinsulin with correct folding and biological activity.	[35]
4.	Antithrombin	Pig	Increased survival rates and avoidance of intravascular coagulation in transplantation models.	[36]
5.	C1-INH	Rabbit	Clinical trials demonstrated that it was effective in lowering the frequency and severity of angioedema attacks.	[37]
6.	Human α-1 antitrypsin	Mice	High biologically active alpha 1 antitrypsin levels were achieved, making them acceptable for therapeutic application.	[38]

7.	Beta-lactoglobulin free milk	Cow	Milk was successfully manufactured without beta-lactoglobulin, considerably lowering allergenicity.	[39]
8.	Lactoferrin	Cow	High expression levels were attained, but the protein retained its functional features.	[40]
9.	Lysozyme	Cow	Lysozyme has high expression levels and retains its antimicrobial action.	[41]
10.	B- and κ-casein	Cow	Enhanced milk composition with higher protein content improves nutritional quality.	[42]
11.	Human clotting factor - IX	Pig	The effective generation of biologically active human factor IX suggests a potential treatment for hemophilia B.	[43]
12.	Factor-VIII	Pig	Human factor VIII that is biologically active and can be used to treat haemophilia A was produced.	[44]
13.	Human serum albumin	Mice	In mice, substantial quantities of human serum albumin were detected within the mammary glands, and the protein demonstrated its expected functional characteristics.	[45]
14.	Plasminogen	Mice	Human tissue plasminogen activator was successfully synthesised and has retained fibrinolytic function.	[46]

CONCLUSION

Genetically modified milk derived from transgenic animals demonstrates substantial advances in biotechnology and therapeutic protein production. The successful manufacture of antibacterial medicines such recombinant human beta defensin-3 in goat milk, as well as human proteins including proinsulin, antithrombin (ATryn), and alpha 1 antitrypsin in cattle and mouse milk, demonstrates the technology's promise. Innovations in hypoallergenic milk and large-scale manufacture of human lactoferrin, lysozyme, factor IX, and factor VIII highlight the advantages of this approach. Comparative assessments demonstrate that transgenic animal systems are more efficient and cost-effective for biopharmaceutical manufacturing. These discoveries indicate a promising future for genetically altered milk in medical and biotechnological applications, providing scalable options for generating important therapeutic proteins. Future research should focus on issues such as protein uniformity and safety, genetic engineering process optimisation, and regulatory barriers. Interdisciplinary collaboration will be critical for progressing this research and turning discoveries into clinically effective medicines. Genetically modified milk is a remarkable biotech innovation with far-reaching consequences for healthcare. It increases the efficiency of biopharmaceutical production and has the potential to greatly improve global health outcomes.

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