Animal Pharming

Use of Transgenic Animals for the Pharmaceutical Production of Human Proteins and Drugs

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Abstract

Animal pharming (also known as gene pharming or molecular pharming) is the production of human pharmaceuticals in farm animals such as cows, sheep, goat, chicken, etc. by genetically manipulating the animal by insertion of a human gene or any other foreign gene *[transgene]* which codes for a particular pharmaceutical. The therapeutic products have a wide range of medicinal uses as vaccines, hormones, defense proteins, monoclonal antibodies which are secreted in the body fluids or tissues of the animal such as milk, blood, urine, semen or eggs, which can then be collected, purified and used as a pharmaceutical product. The first transgenic farm animal was Tracy the sheep, in 1985, which expressed high levels of human protein alpha-1-anitrypsin.

This article delves into the composite methods employed in pharming, the applications and advantages and the commercial aspects. The operations include DNA microinjection, Retrovirus mediated gene transfer, Embryonic Stem cell mediated gene transfer and more recently cloning methods, each one having its own advantage. Pharming is useful in targeted production of pharmaceutical proteins and drugs, producing simple and complex human proteins in animals, which cannot be produced by traditional r-DNA coupled cell culture method. Finally, this article reflects on the advantages over traditional cell culture method, difficulties and initial high cost involved in producing a transgenic animal, and, once obtained, the ease of producing a large population in a short time by the virtue of cloning.

Keywords: Gene pharming, Transgene, Transgenic animal, r-DNA

1. Introduction

Animal pharming, also known as gene pharming or molecular pharming is the production of human pharmaceuticals in farm animals such as cows, sheep, goat, chicken, etc. by genetically manipulating the animal by insertion of a human gene or any other foreign gene *[transgene]* which codes for a particular pharmaceutical. The therapeutic products have a wide range of medicinal uses as vaccines, antibiotics, hormones, defence proteins, which are secreted in the body fluids or tissues of the animal such as milk, blood, urine, semen or eggs, which can then be collected, purified and used as a pharmaceutical product.

2. Techniques employed for obtaining transgenic animals

The universality of the genetic code permits the polymerases of one organism to transcribe for a gene from another organism. This general rule of the nature enables developing a transgenic animal possible.

The basic step for production of a transgenic animal is the *in vitro* preparation of the gene construct containing the transgene, which codes for a particular protein or drug, by using the techniques of genetic engineering. The gene construct is then further exploited by using any of the methods described below.

2.1 **Pronuclear DNA Microinjection:** In this method, the gene construct is directly microinjected into the pronucleus [*nucleus of ovum or sperm after fertilization but before union to form zygote*] of a fertilized ovum. The manipulated fertilized ovum is then transferred into the uterus of a foster mother, which gives birth to a transgenic animal.

However, in this method, the insertion of gene is a random process and there is a high probability of over or under-expression of certain genes and a complete non-expression of the transgene. The major advantage of this method is its applicability to a wide variety of species. See Fig. 1

2.2 **Retrovirus / Lentivirus Mediated Gene Transfer:** In this method, the gene construct is transferred to the host cell by means of a vector, which is a retrovirus, taking advantage of its ability to infect host cells. The result is a chimeric animal, which has only a few cells with manipulated DNA. To obtain a complete transgenic animal, the chimeric animals are then inbred for as many as 20 generations.

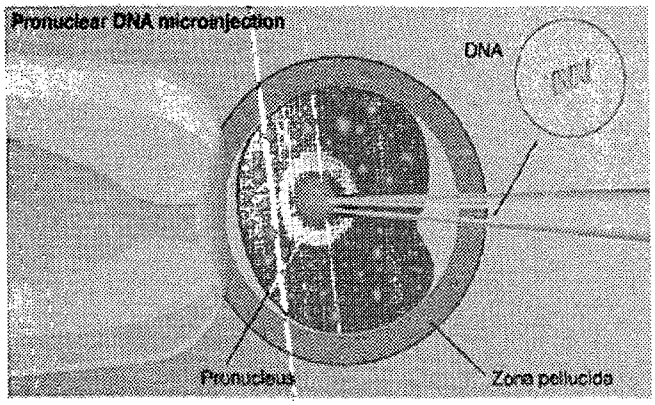

Fig.1- Pronuclear DNA Microinjection
Source: Nature Magazine website: www.nature.com/.../v5/n1/fig_tab/7400053_f1.html (accessed Nov 2007)

If the retrovirus infects the germ cells in the embryo, the resulting animal would be a complete transgenic animal. This method shows increased probability of gene expression. See Fig.2

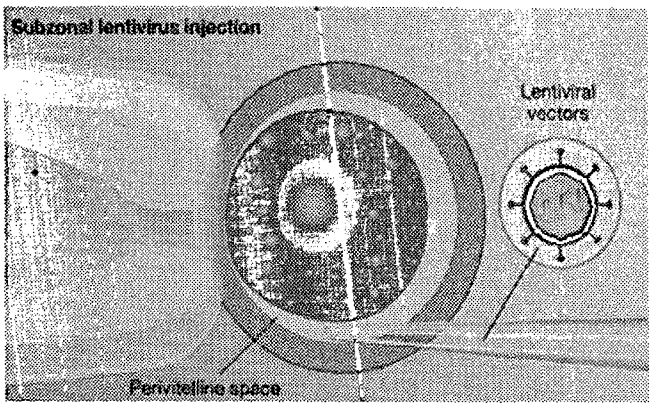

Fig.2- Lentivirus mediated gene transfer
Source: Nature Magazine website: www.nature.com/.../v5/n1/fig_tab/7400053_f1.html (accessed Nov 2007)

2.3 Embryonic Stem Cell Mediated Gene Transfer: In this method, the totipotent stem cells are isolated from the developing embryo of the animal. Stem cells are undifferentiated cells which have the potential to develop into any type of cell. The *in vitro* culture of stem cells is infected with the gene construct. The stem cells with the incorporated gene construct is then inserted in an embryo at the blastocyst stage. The result is a chimeric animal with mixed DNA.

The presence of the transgenes can be tested at the embryonic stage in this method. See Fig.3

2.4 Nuclear Transfer with Somatic cells or Embryonic Stem cells or Embryonic Germ cells [Cloning]: Cloning requires a single cell with a complete set of chromosomes. The cell can either be a somatic cell or an embryonic stem cell. In somatic cell nuclear transfer, the nucleus of an egg cell is sucked out with a micropipette. A somatic cell is then carefully inserted into the perivitelline space of the enucleated egg cell. The assembly is subjected to electric shock to facilitate the penetration of the somatic cell into the egg cell. The somatic cell nucleus is reprogrammed by the host cell. The egg, now containing the somatic cell, is stimulated with a shock and will

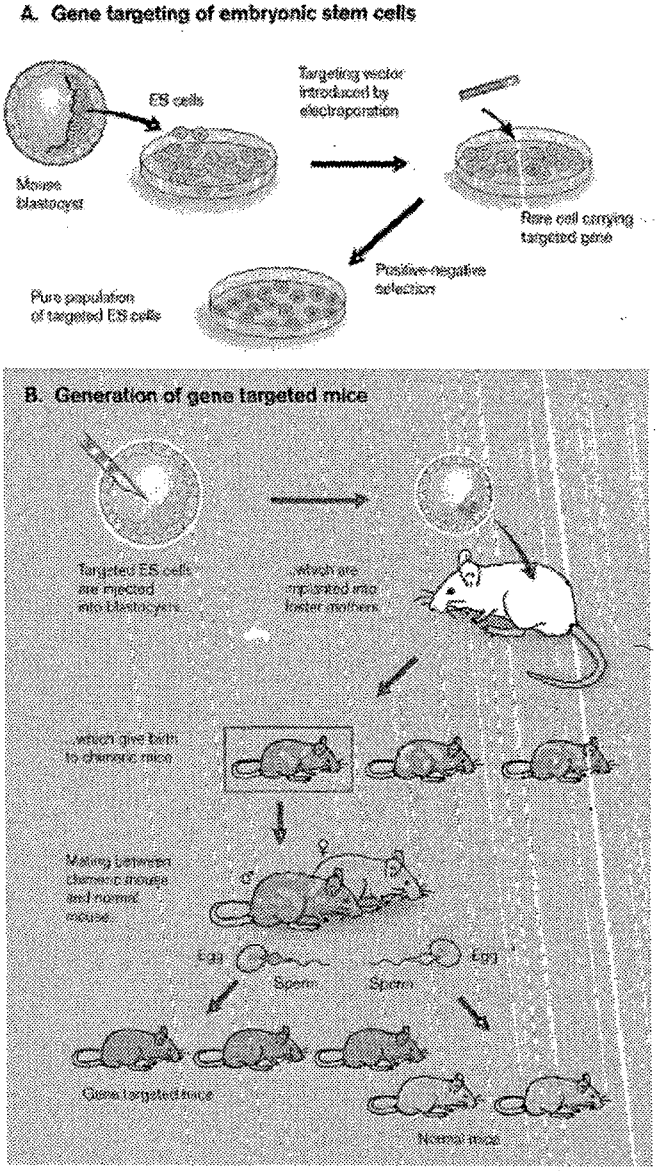

Fig.3- Embryonic Stem cell mediated gene transfer
Source: Website of Nobel Prize Organization nobelprize.org/.../laureates/2007/adv.html (accessed Nov 2007)

begin to divide. After many mitotic divisions in culture, this single cell forms a blastocyst (an early stage embryo with about 16-20 cells) which is then implanted in the womb of foster mother for gestation. This technique is currently the basis for cloning animals. The same technique can be employed for transgenic animal development by using a transgenic cell, instead of a somatic cell. As for transgenesis, embryonic stem cell nuclear transfer is more specific to gene targetting. Currently, as cloning using this method is crossing success rate of 30% (Ref.5), the traditional methods used since 1985 for obtaining transgenic animals are taking a backseat. See Fig.4

Other Methods for developing Transgenic Animals for Pharming:

2.5 Sperm mediated exogenous DNA transfer during In vitro Fertilization

2.6 Liposome mediated DNA transfer

2.7 Electroporation of DNA into sperm, ova or embryos

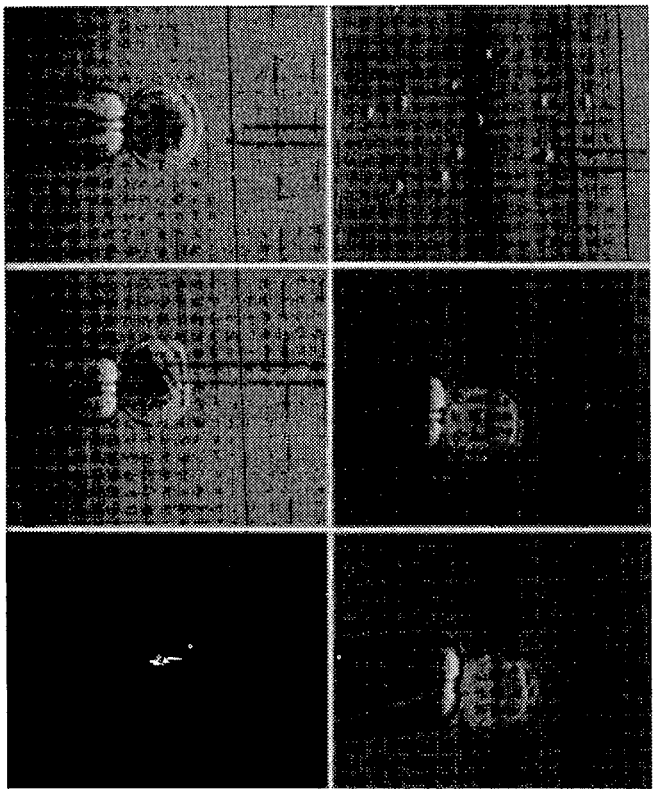

Fig.4- Somatic Cell Nuclear Transfer
Source: Photo from Roslin Institute, UK http://www.roslin.ac.uk/imagelibrary/popups/110.php (accessed Nov 2007)

2.8 Biolistics [Use of a gene gun to 'shoot' DNA into the animal cells in vitro]
2.9 Testis injection with DNA to produce transgenic spermatogenic stem cells
2.10 Male Germ Cell Transplantation

Until recently, pronuclear microinjection of deoxyribonucleic acid (DNA) was the standard method for producing transgenic animals. This technique is now being replaced by more efficient protocols based on somatic nuclear transfer that also permit targeted genetic modifications. Lentiviral vectors and small interfering ribonucleic acid technology are also becoming important tools for transgenesis.

3. Applications

- Some of the pharmaceuticals that can be obtained by animal pharming are listed in Table 1.
- Pharming products currently in development are listed in Table 2.

4. Advantages over traditional cell culture method

- Cell and bacterial cultures require constant monitoring and sampling.
- Expansion is more costly, because substantial plant machinery must be purchased and maintained.
- Isolating and purifying proteins is more difficult than purifying proteins from an animal's milk or bodily fluid.
- Animals as bioreactors are more cost-efficient as product is effectively passed through milk, with an average of 53% drug with 99% purity. Purifying process more simple when drug obtained in poultry eggs and urine.
- Animals naturally carry the complex cellular mechanisms to transcribe complex proteins, something, which is difficult, or may be impossible to replicate in cell culture microbes.
- The unit cost per protein is significantly reduced when equated to traditional cell culture method as requirement for complex machinery and its maintenance is obviated. A private firm's

Table 1-Pharmaceuticals from Animal Pharming

Animal	Drug / Protein	Use
Cow	alpha-lactalbumin	Anti-infection
Cow	factor VIII	Treatment of Hemophilia
Cow	fibrinogen	Wound healing
Cow	collagen I, collagen II	Tissue repair, treatment of rheumatoid arthritis
Cow	lactoferrin	Treatment of GI tract infection & infectious arthritis
Cow	human serum albumin	Maintains blood volume
Goat	human protein C	Treatment of Thrombosis
Goat	antithrombin 3	Treatment of Thrombosis
Goat	glutamic acid decarboxylase	Treatment of Type I diabetes
Goat	Pro 542	For HIV infection
Sheep	α_1 anti-trypsin	Emphysema
Sheep	CFTR	Treatment of Cystic fibrosis
Sheep	tissue plasminogen activator	Treatment of Thrombosis
Sheep	factor VIII, IX	Treatment of Hemophilia
Sheep	fibrinogen	Treatment of wound healing
Pig	tissue plasminogen activator	Treatment of Thrombosis
Pig	factor VIII, IX	Treatment of Hemophilia
Pig	insulin	Diabetes Mellitus
Chicken, Cow, Goat	Monoclonal Antibodies	Other vaccine production

Source: (Reference 21) United Stated Department of Agriculture: Animal and Plant Health Inspection Service
website: http://www.aphis.usda.gov/vs/ceah/cei/bi/emergingmarketcondition_files/animal_pharming.htm (accessed Nov 2007)

Table 2- Pharming products currently in development

Product	Purpose	Phase
Recombinant human C_1 inhibitor [rhC$_1$INH]	Hereditary Angioedema	Phase III
Recombinant human C_1 inhibitor [rhC$_1$INH]	Other indications	Phase I
Recombinant tissue sealant [rTS]	Trauma and Surgery	Pre-clinical
Recombinant human collagen type I [rhCOL]	Intermediate for medical devices and aesthetic products	Pre-clinical
Recombinant human lactoferrin (hLF)	Infection and inflammatory diseases, pharmaceutical and nutraceutical	Phase I completed
ATryn	Prevent blood clotting in genetic disease	Approved by EMEA in 2007 [European Medicines Agency]

Source: (Reference 10, 24) Scott, A., EMEA approves first pharmed drug, Chemical Week 168 (33) (2006)
Website of Pharming Group N.V., a company developing transgenic animals for pharming. www.pharming.com (accessed Dec 2007)

estimation for comparative cost study depicts the superiority of transgenic animals for production of human pharmaceuticals:

AviGenics comparison of production inputs and costs for monoclonal antibodies using traditional cell culture versus using transgenic poultry or goats is shown in Table 3 below.

	Traditional Cell Culture	Poultry Eggs	Goat Milk
Raw Material Volume (kg)	170,000	250	21,000
Capital Equipment Cost, or Cost per Animal (dollars)	100 Million	1,000	10,000 to 50,000
Equipment Maintenance Costs, or Keeping Cost per Animal (dollars)	100,000	10	2,500
Unit Cost per Protein (dollars per gram)	100	0.10 - 0.25	2 to 20

Source: (Reference 21) United States Department of Agriculture: Animal and Plant Health Inspection Service
website: http://www.aphis.usda.gov/vs/ceah/cei/bi/emergingmarketcondition_files/animal_pharming.htm (accessed Nov 2007)

5. Scope

- According to a report by 'Animal and Plant Health Inspection Service-United States Department of Agriculture', an estimation by a Wisconsin based firm that clones transgenic cows for pharmaceutical production states that one transgenic animal, in its lifetime, can produce $200 to $300 million worth of pharmaceuticals. This report distinctly depicts the potential impact of pharming on the medical world.
- The world market is growing for human pharmaceutical products. By the end of 2006, almost 170 recombinant therapeutic proteins or antibody-based products had gained approval in either the US or the EU, commanding an estimated global market value of $40 billion (ref.28). With the success rate of cloning touching 30% (ref.5) and advances in cloning techniques, the difficulties and initial high investment currently involved are likely to be resolved, as cloning would enable production of a large population of pharming animals in a short time. But the ethical debates would continue to persist, necessitating regulatory constraints, and essentially so.

6. References

1. Dr. Pradeep Parihar, *A Textbook of Biotechnology*, Student Edition, **116.**
2. Sandhya Mitra, *Genetic Engineering: Principles & Practice*, **185.**
3. S.K.Sopory, *Biotechnology Applications and Careers*, **52.**
4. Pinkert, *Transgenic Animals Technology*, **121, 173, 396.**
5. *'Dolly for Dinner? Assessing Commercial & Regulatory Trends in Cloned Livestock'*, Nature Biotechnology (25) (1) (Jan 2007), **48-49.**
6. *Trends in Biotechnology*, Vol. 25, Number 5, **189-193, 205.**
7. 'Transgenic Animals in the Production of Therapeutic Proteins', Biotechnology International. Century Press, 1992, **317.**
8. Niemann, H., Kues, W.A., 'Transgenic farm animals: An update', *Reproduction, Fertility and Development* 19 (6) (2007), pp. **762-770.**
9. Pfeifer, A., 'Lentiviral transgenesis - A versatile tool for basic research and gene therapy', *Current Gene Therapy* 6 (4) (2006), pp. **535-542.**
10. Scott, A., 'EMEA approves first pharmed drug' *Chemical Week* 168 (33) (2006).
11. Bauman, D.E., Mather, I.H., Wall, R.J., Lock, A.L., 'Major advances associated with the biosynthesis of milk', *Journal of Dairy Science* 89 (4) (2006), pp. **1235-1243.**
12. Niemann, H., Kues, W., Carnwath, J.W., 'Transgenic farm animals: Present and future', *OIE ,Revue Scientifique et Technique* 24 (1) (2005), pp. **285-298.**
13. Edwards, J.L., Schrick, F.N., McCracken, M.D., Van Amstel, S.R., Hopkins, F.M., Welborn, M.G., Davies, C.J., 'Cloning adult farm animals: A review of the possibilities and problems associated with somatic cell nuclear transfer', *American Journal of Reproductive Immunology* 50 (2) (2003), pp. **113-123.**
14. Wall, R.J.,' Biotechnology for the production of modified and innovative animal products: Transgenic livestock bioreactors', *Livestock Production Science* 59 (2-3) (1999), pp. **243-255.**
15. Pennisi, E., 'After Dolly, a pharming frenzy' *Science* 279 (5351) (1998), pp. **646-648.**
16. Nasto, B., 'Pharming seeks public support', *Nature Biotechnology* 16 (9) (1998), pp. **807-808.**
17. Seppanen, P., 'Pharming looks to pharmaceutical production through new biotechnology', *Kemia-Kemi/Finnish Chemical Journal* 24 (3) (1997), pp. **193-195.**
18. Wall, R.J., Kerr, D.E., Bondioli, K.R., 'Transgenic Dairy Cattle: Genetic Engineering on a Large Scale', *Journal of Dairy Science* 80 (9) (1997), pp. **2213-2224.**
19. Sendai, Y., Hoshi, H., 'Gene targeting of animals', *Nippon Nogeikagaku Kaishi* 70 (8) (1996), pp. **907-910.**
20. Bialy, H., 'Transgenic pharming comes of age', *Bio/Technology* 9 (9) (1991), pp. **786-788.**
21. United States Department of Agriculture: Animal and Plant Health Inspection Service website: http://www.aphis.usda.gov/vs/ceah/cei/bi/emergingmarketcondition_files/animal_pharming.htm (accessed Nov 2007).
22. Office of Biotechnology, Iowa state university website: http://www.biotech.iastate.edu/biotech_info_series/bio10.html (accessed Nov 2007)
23. The University of Utah website: http://learn.genetics.utah.edu/features/pharming/ (accessed Dec 2007)
24. Website of Pharming Group N.V., a company developing transgenic animals for pharming. www.pharming.com (accessed Dec 2007).
25. Department of Biochemistry and Molecular Biophysics, The University of Arizona website: http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture20/ (accessed Dec 2007) .
26. http://www.drugdeliverytech-online.com/drugdelivery/200704/?pg=47 (accessed Dec 2007).
27. Biotechnology Australia Government Website http://www.biotechnologyonline.gov.au/human/pharming.cfm (accessed Dec 2007).
28. Bio Pharm International magazine website http://www.biopharminternational.com/biopharm/Upstream+Processing/Engineering-Biopharmaceuticals/ArticleStandard/Article/detail/470171?searchString=engineering%20biopharmaceuticals (accessed Nov 2007).