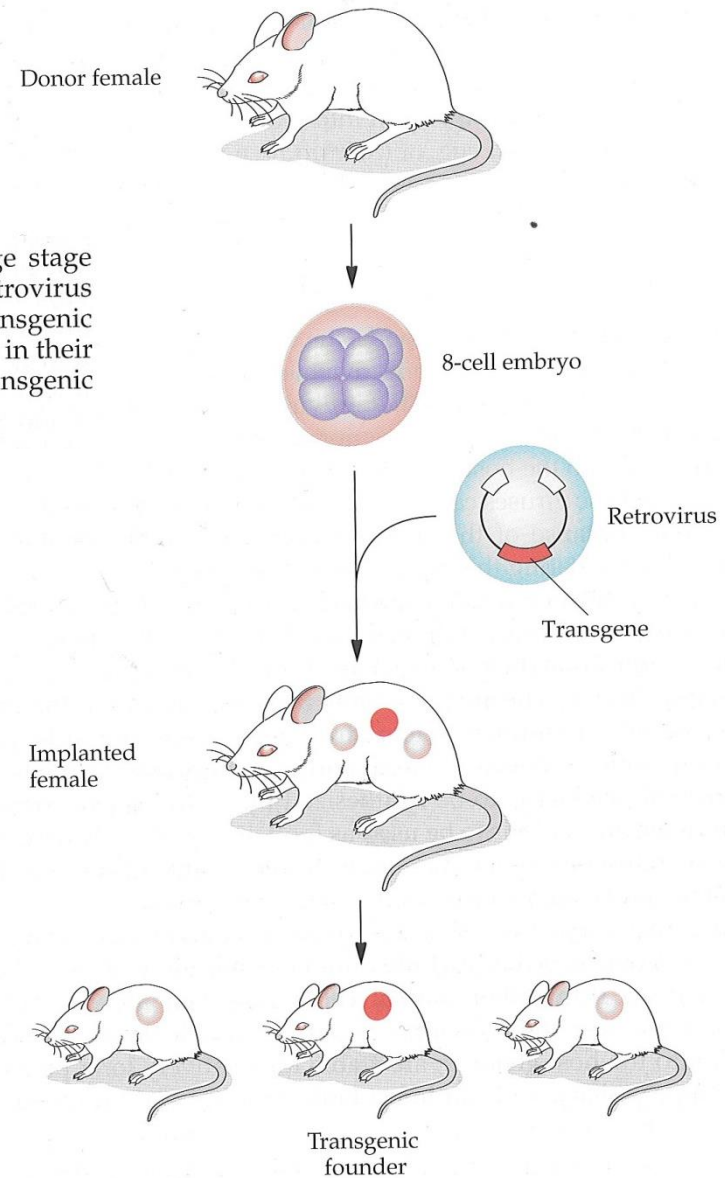
A white line-art illustration of a large, multi-story building with many windows and a central entrance, set against a dark grey background.

MOL.911
Transgenic Animals

Transgenic Animals

FIGURE 21.1 Establishing transgenic mice with retroviral vectors. Cleavage stage embryos, usually at the eight-cell stage, are infected with a defective retrovirus carrying a transgene. Implanted females (foster mothers) give birth to transgenic pups. Matings are carried out to determine which pups have the transgene in their germ line cells. Transgenic lines can be established from these founder transgenic animals.



Transgenic Animals

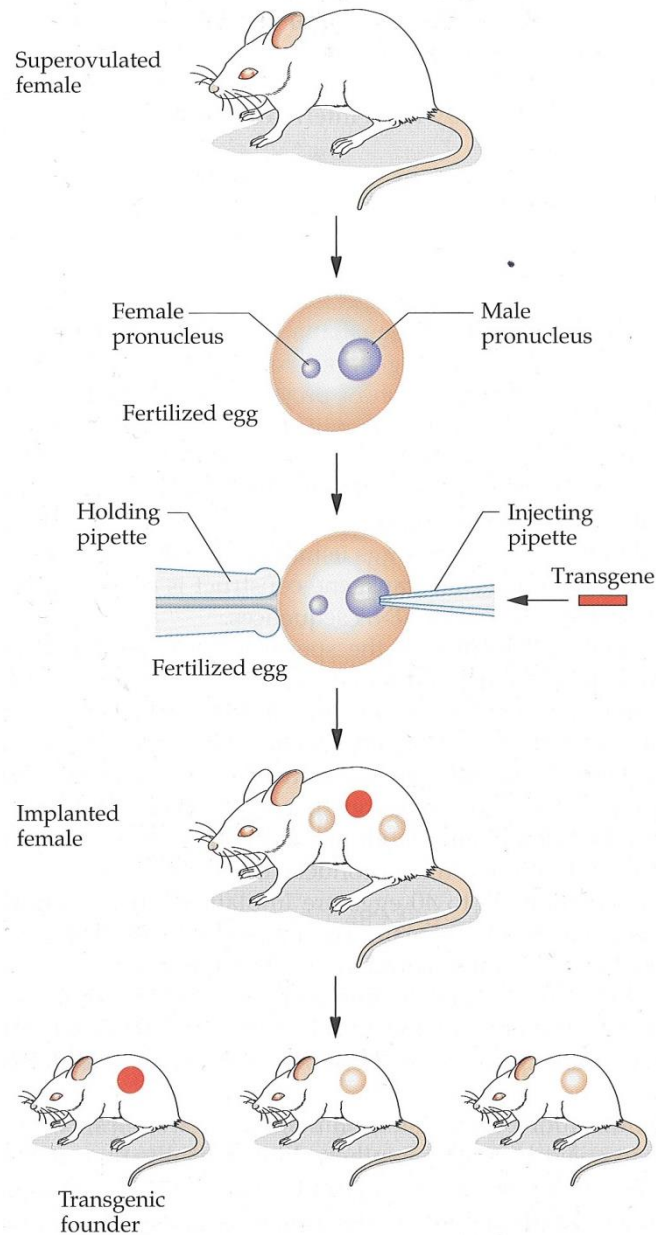


FIGURE 21.3 Establishing transgenic mice by DNA microinjection. Eggs are obtained from donor females that have been induced to superovulate and then mated with males. Purified samples of the transgene construct are microinjected into the male pronucleus of a fertilized egg. Implanted females (foster mothers) give birth to transgenic pups, from which transgenic lines can be established.

Transgenic Animals

4

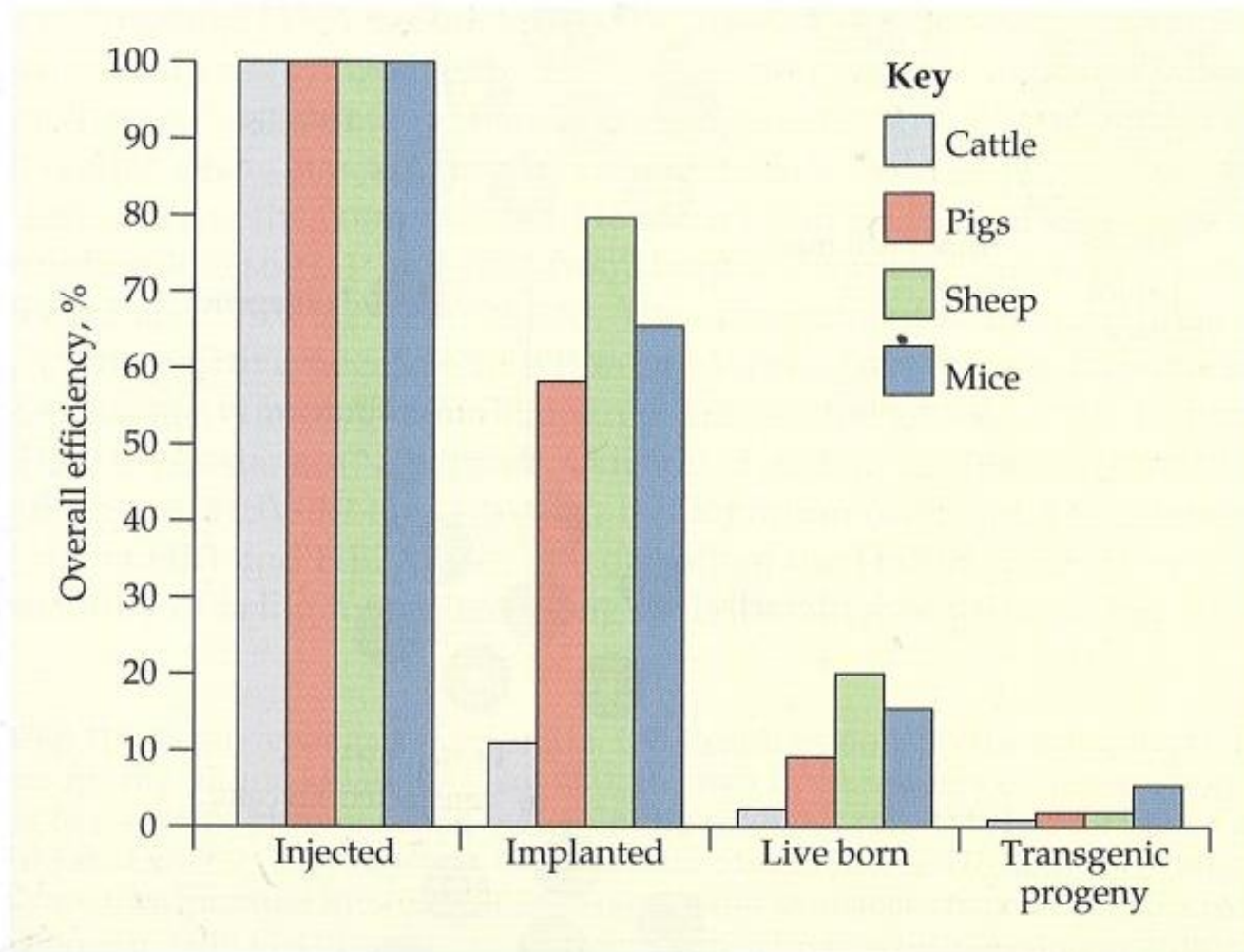


FIGURE 21.4 Overall efficiency of the transgenesis process after DNA microinjection. All the fertilized eggs (100%) of cattle, pigs, sheep, and mice are inoculated with a transgene, but the success of implantation and giving birth to offspring is much lower, and only 5% or fewer of the treated eggs become transgenic progeny.

5

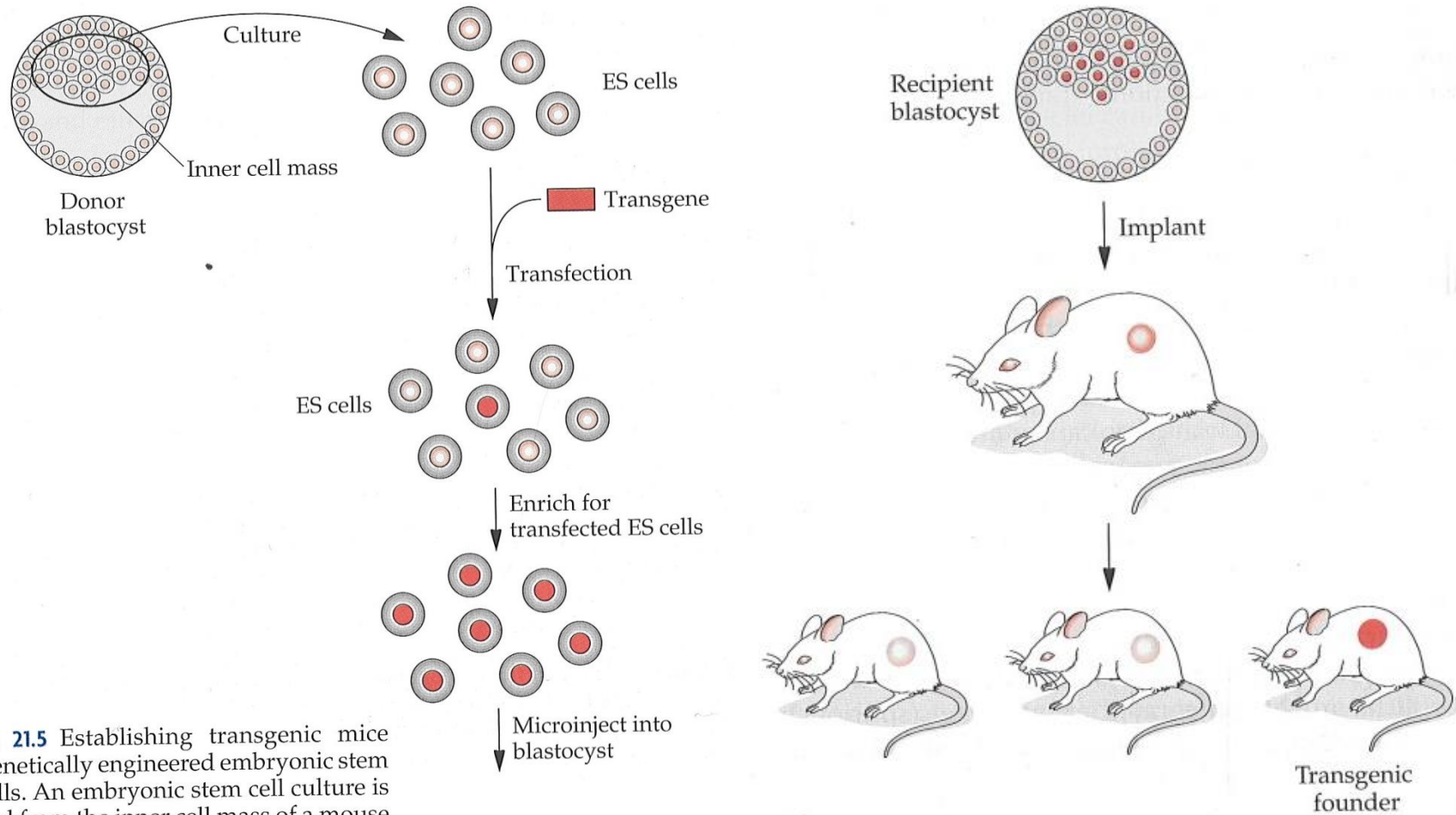


FIGURE 21.5 Establishing transgenic mice with genetically engineered embryonic stem (ES) cells. An embryonic stem cell culture is initiated from the inner cell mass of a mouse blastocyst. The embryonic stem cells are transfected with a transgene. After growth, the transfected cells are identified by either the positive-negative selection procedure or PCR analysis. Populations of transfected cells can be cultured and inserted into blastocysts, which are then implanted into foster mothers. Transgenic lines can be established by crosses from founder mice that carry the transgene in their germ lines.

Transgenic Animals

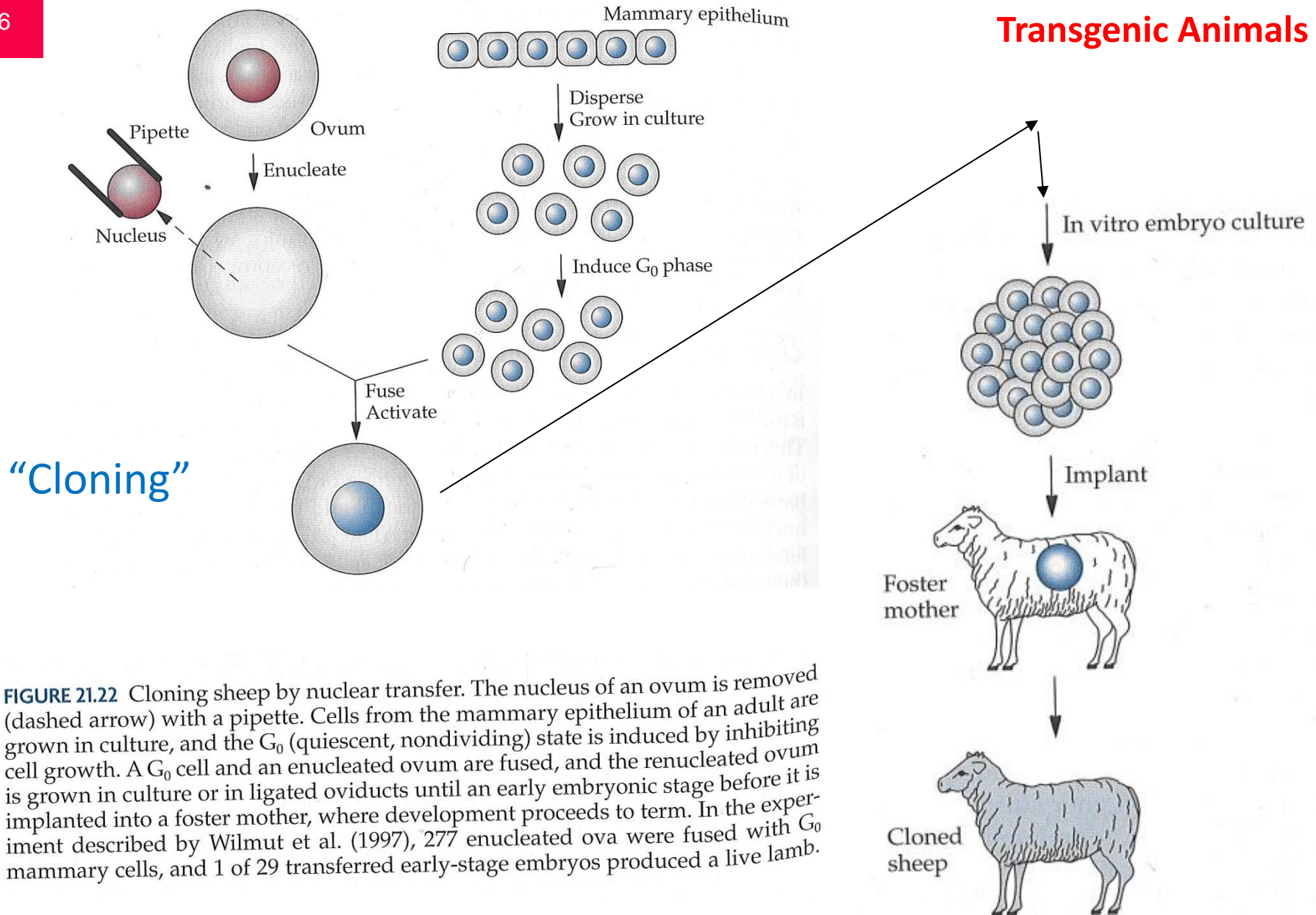


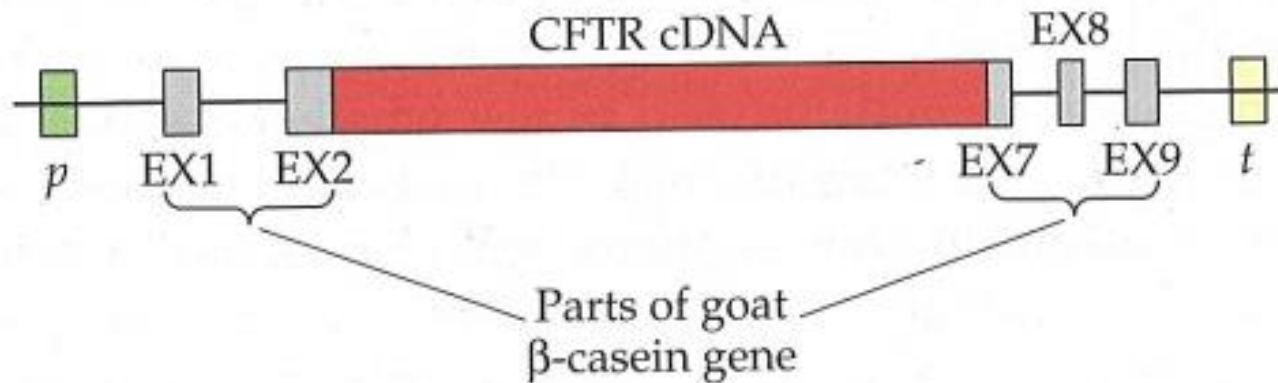
FIGURE 21.22 Cloning sheep by nuclear transfer. The nucleus of an ovum is removed (dashed arrow) with a pipette. Cells from the mammary epithelium of an adult are grown in culture, and the G₀ (quiescent, nondividing) state is induced by inhibiting cell growth. A G₀ cell and an enucleated ovum are fused, and the renucleated ovum is grown in culture or in ligated oviducts until an early embryonic stage before it is implanted into a foster mother, where development proceeds to term. In the experiment described by Wilmut et al. (1997), 277 enucleated ova were fused with G₀ mammary cells, and 1 of 29 transferred early-stage embryos produced a live lamb.

Transgenic Animals

TABLE 21.2 Some human proteins that have been expressed in the mammary glands of transgenic animals

Antithrombin III
α_1 -Antitrypsin
Calcitonin
Erythropoietin
Factor IX
Factor VIII
Fibrinogen
Glucagon-like peptide
α -Glucosidase
Granulocyte colony-stimulating factor
Growth hormone
Hemoglobin
Serum albumin
Insulin
Insulin-like growth factor 1
Interleukin 2
α -Lactalbumin
Lactoferrin
Lysozyme
Monoclonal antibodies
Nerve growth factor
Protein C
Superoxide dismutase
Tissue plasminogen activator

FIGURE 21.24 Goat β -casein gene–CFTR cDNA expression construct. The full-length cDNA for CFTR was cloned between exon 2 (EX2) and exon 7 (EX7) of the goat β -casein gene. The promoter (*p*) and transcription termination (*t*) sequences and exons 1, 8, and 9 (EX1, EX8, and EX9) of the β -casein gene were retained.



CFTR: Cystic Fibrosis Transmembrane Conductance Regulator

Table 19.2 Mammary gland transgenes, promoter sequences, and recipient organisms

Transgene	Promoter	Transgenic species
Longer-acting tissue plasminogen activator	Whey acidic protein	Goat
α_1 -Antitrypsin	β -Lactoglobulin	Sheep
Clotting factor IX	β -Lactoglobulin	Sheep
Soluble CD4 protein	Whey acidic protein	Mouse
Lactoferrin	α_{s1} -Casein	Cattle
Urokinase	α_{s1} -Casein	Mouse
CFTR	β -Casein	Mouse
Interleukin-2	β -Casein	Rabbit

Table 4 Expression of Recombinant Human Proteins in the Milk of Transgenic Pigs

Protein	Promoter	Expression Level	Company	Reference
hPC	Mouse WAP	1 mg/mL	GTC Biotherapeutics	Velander et al. (1992)
hPC	Ovine BLG	0.75 mg/mL	PPL Therapeutics	PPL literature
hFVIII	Mouse WAP	3 µg/mL		Paleyanda et al. (1997)
hEPO	Mouse WAP	878 IU/mL		Park et al. (2006)

Table 5 Expression of Recombinant Human Proteins in the Milk of Transgenic Sheep

Protein	Promoter	Expression Level	Company	Reference
h α_1 AT	Ovine BLG	35 mg/mL		Wright et al. (1991)
hFVII	Ovine BLG	2 mg/mL	PPL Therapeutics	PPL literature
hFVIII	Ovine BLG	6 ng/mL		Niemann et al. (1999)
hFIX	Ovine BLG	25 ng/mL		Simons et al. (1988)
hFIX	Ovine BLG	5 ng/mL		Clark et al. (1989)
hFIX	Ovine BLG	1.0 mg/mL	PPL Therapeutics	Schnieke et al. (1997)
hFIB	Ovine BLG	5.0 mg/mL	PPL Therapeutics	Garner and Colman (1998)
hFIB	Ovine BLG	5 mg/mL		Butler et al. (1997)
hPC	Ovine BLG	0.3 mg/mL	PPL Therapeutics	Garner and Colman (1998)

Table 6 Expression of Recombinant Human Proteins in the Milk of Transgenic Goats

Protein	Promoter	Expression Level	Status	Company	Reference
htPA	Murine WAP	3 μ g/mL 610,000 IU/ mg			Ebert et al. (1991), Denman et al. (1991)
htPA	Goat β -casein	3 mg/mL (6 mg/mL?)			Ebert et al. (1994)
hAT-III	Goat β -casein	20 mg/mL	ATryn [®] EU: approved US: prelaunch	GTC Biotherapeutics/LEO Pharma	Genzyme Transgenics (1996), GTC literature Edmunds et al. (1998) Baguisi et al. (1999)
hAT-III	Goat β -casein	5.8 mg/mL			
hGH	Retrovirus	60 ng/mL			Archer et al. (1994)
hGH	Adenovirus	0.3 mg/mL			Sanchez et al. (2004)
h α_1 AT	Goat β -casein	14 mg/mL		GTC Biotherapeutics	Genzyme Transgenics (1996), GTC literature
h α FP	Goat β -casein		Phase II (2004)	GTC Biotherapeutics/Merrimack Pharmaceuticals	Parker et al. (2004), http://www.transgenics.com/products/novel.html , http://www.clinicaltrials.gov/ct/show/NCT00147329?order=1
hEPO	Adenovirus	2 mg/mL			Toledo et al. (2006)
hLF	Adenovirus	2.6 mg/mL			Han et al. (2007)

Table 7. Expression of Recombinant Human Proteins in the Milk of Transgenic Cattle

Protein	Promoter	Expression Level	Method	Status	Company	Reference
h α -LA		2.4 mg/mL				Krimpenfort et al. (1991)
h α -LA	Human α -LA	2.4 μ g/mL?	Microinjection	Preclinical	PPL Therapeutics	PPL literature
hCOL				Preclinical	Pharming	
hFIB	Bovine α_{S1} -casein	3 mg/mL	Nuclear transfer	Preclinical	Pharming	
hGH	Bovine α_{S1} -casein	5 mg/mL	Nuclear transfer		Bio Sidus SA, Buenos Aires, Argentina	Salamone et al. (2006)
hLF	Bovine α_{S1} -casein		Microinjection		PPL Therapeutics	McKee et al. (1998)
hLF	Bovine α_{S1} -casein	2.8 mg/mL	Microinjection	Phase I completed	Pharming	van Berkel et al. (2002)
hSA				In development	GTC Biotherapeutics/TransOva Genetics	GTC literature

TABLE 6: Recombinant proteins produced from transgenic rabbits.

Expressed proteins	Promoter	Expressed protein	References
Human α 1-antitrypsin	Human α 1-antitrypsin DNA	1 g/L in plasma	[17]
Human α -glucosidase	Bovine α s1-casein	8 g/L	[37]
	N-acetyl- β -glucosaminyl	NA	[38]
Human CI inhibitor	NA	NA	[39]
Human clotting factor VIII	Mouse WAP	NA	[40]
	Mouse WAP	0.005–0.161 g/L	[41]
Human erythropoietin	Rabbit WAP	0.0000003 g/L	[42]
	Rabbit WAP	NA	[43]
	Bovine β -lactoglobulin	0.5 g/L	[44]
	Rabbit WAP	NA	[45]
	Rabbit WAP	60–178 IU/L	[46]
Human extracellular SOD	Mouse WAP	3 g/L	[47]
Human growth hormone	Mouse WAP	0.000012 g/L	[48]
	Rat WAP	0.5–1.0 g/L	[49]
	Rat WAP	0.010 g/L	[50]
Human IL-2	Rabbit β -casein	0.0005 g/L	[51]
Human insulin-like growth factor	Bovine α s1-casein	1 g/L	[52]
	Bovine α s1-casein	0.3 g/L	[53]
	Bovine α s1-casein	0.678 g/L	[54]
Human nerve growth factor β	Bovine α s1-casein	0.25 g/L	[55]
	Adenoviral	0.346 g/L	[56]
Human tPA	Bovine α s1-casein	0.00005 g/L	[57]
Bovine chymosin	Bovine α s1-casein	1.5 g/L	[58]
Bovine FSH	Bovine α s1-casein	0.1 g/L	[59]
Equine chorionic gonadotropin	Rabbit WAP	0.022 g/L	[60]
Salmon calcitonin	Ovine β -lactoglobulin	2.1 g/L	[61]
Human protein C	Mouse WAP	0.0000001–0.0000003 g/L	[62]
TNAP	Human WAP	NA	[63]
Human lactoferrin	Adenoviral	2.3 g/L	[64]
Human interferon beta	NA	2.2–7.2 \times 10 ⁷ IU/L	[65]
Human antithrombin	Adenoviral	4.8 g/L	[66]

FSH: follicle stimulating hormone; IL-2: interleukin-2; NA: not available; SOD: superoxide dismutase; TNAP: tissue-nonspecific alkaline phosphatase; tPA: tissue plasminogen activator; WAP: whey acidic protein.

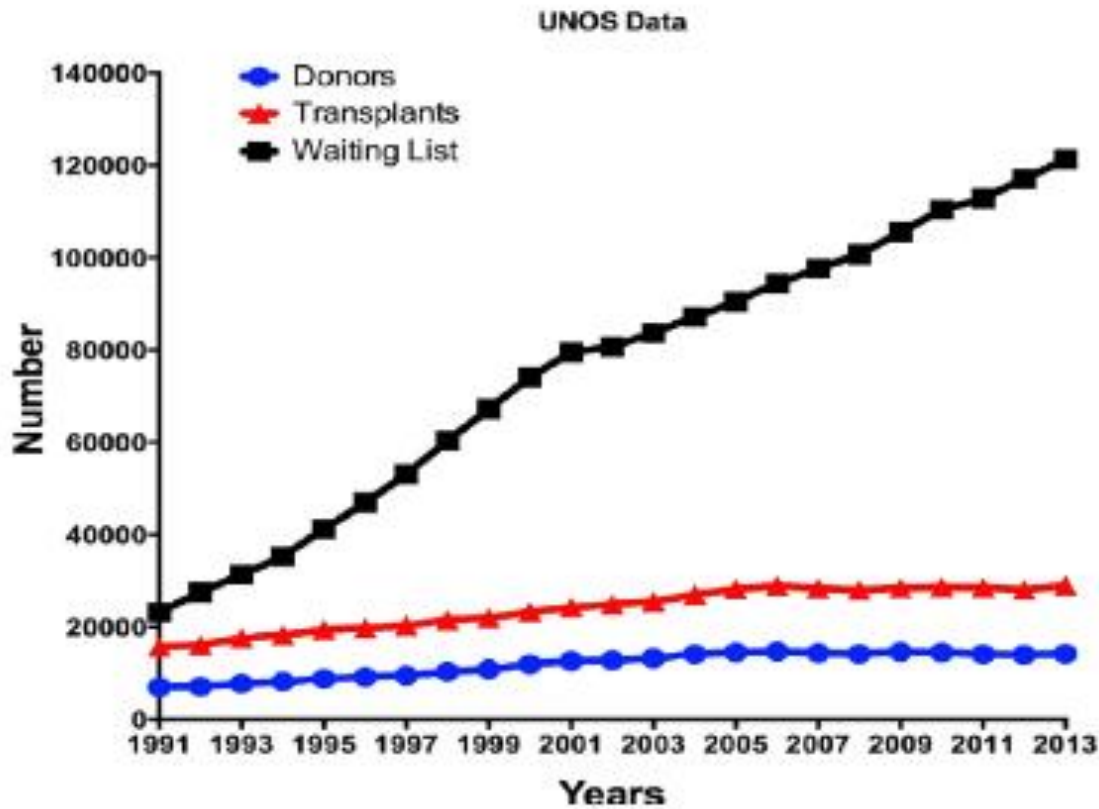
Table 1 Demand of Some Human Proteins for Clinical Use in the United States and the Estimated Number of Livestock Animals Needed for Production Calculated with an Average Recombinant Protein Expression of 1 g/L of Milk

	Factor VIII(hfVIII)	Factor IX(hFIX)	Protein C(hPC)	Antithrombin III(hAT-III)	Fibrinogen(hFib)	Albumin(hAlb)	Annual Milk Yield (L)
Amount needed (kg/year)	0.3	4	10	21	150	315,000	
Rabbit	60	800	2,000	4,200	30,000	63,000,000	5
Pig	1	14	34	70	500	1,050,000	300
Sheep	1	8	20	42	300	630,000	500
Goat	1	5	13	27	188	393,750	800
Cattle	1	1	2	3	19	39,375	8,000

Table 2 Parameters to Be Considered When Choosing Animal Species for Transgenic Milk Expression

Species	Milk Yield per Lactation(L)	Gestation(Months)	Maturation(Months)	Elapsed Time from Microinjection to First Lactation (Months)
Rabbit	1–1.5	1	4–6	6–8
Pig	100–300	4	7–8	15–16
Sheep	400–600	5	6–8	16–18
Goat	800–1,000	5	6–8	16–18
Cattle	Up to 10,000	9	12–15	30–33

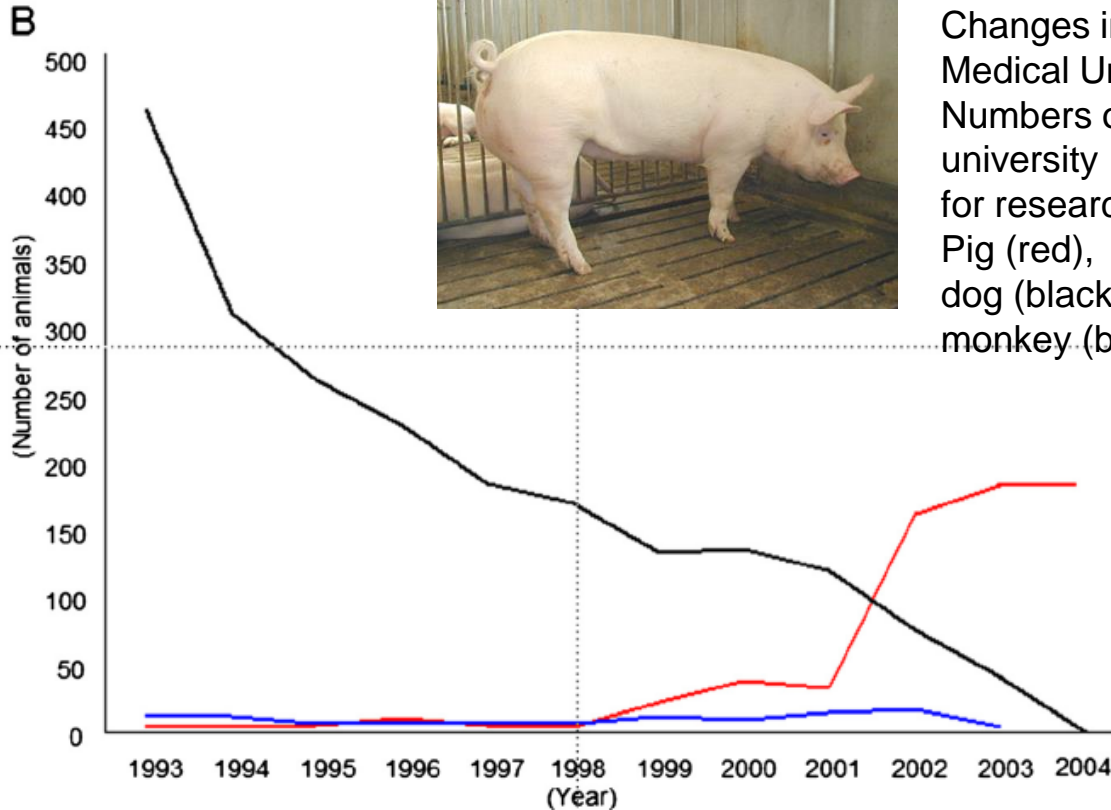
Transgenic animals as organ supplier



Transgenic animals as organ supplier



Changes in use of large animals at Jichi Medical University. A) Domestic pig. B) Numbers of large animals used at our university for research. Pig (red), dog (black), monkey (blue).



Transgenic animals as organ supplier

The advantages and disadvantages of the pig vs baboon as a potential source of organs and cells for humans.

	Pig	Baboon
Availability	Unlimited	Limited
Breeding potential	Good	Poor
Period to reproductive maturity	4–8 months	3–5 years
Length of pregnancy	114 ± 2 days	173–193 days
Number of offspring	5–12	1–2
Growth	Rapid (adult human size within 6 months) ^a	Slow (9 years to reach maximum size)
Size of adult organs	Adequate	Inadequate ¹
Cost of maintenance	Significantly lower	High
Anatomical similarity to humans	Moderately close	Close
Physiological similarity to humans	Moderately close	Close
Relationship of immune system to humans	Distant	Close
Knowledge of tissue typing	Considerable (in selected herds)	Limited
Necessity for blood type compatibility with humans	Probably unimportant	Important
Experience with genetic engineering	Considerable	None
Risk of transfer of infection (xenozoonosis)	Low	High
Availability of specific pathogen-free animals	Yes	No
Public opinion	More in favor	Mixed

^a Breeds of miniature swine are approximately 50% of the weight of domestic pigs at birth and sexual maturity and reach a maximum weight of approximately 30% of standard breeds. At full size, miniature swine are easier to house and to handle. Furthermore, inbred herds are available, though cloning of any pig can result in inbred herds, if needed. Although MHC-identical miniature swine may have some specific immunologic advantage, the disadvantage is that they cannot be cross-bred with other pig strains in which a genetic modification has been introduced; if cross-breeding is carried out, clearly MHC identity is lost. (Reproduced with permission from Cooper DKC. A brief history of cross-species organ transplantation. *Baylor Univ Med Center Proc* 2012; 25:49–57).

Transgenic animals as organ supplier

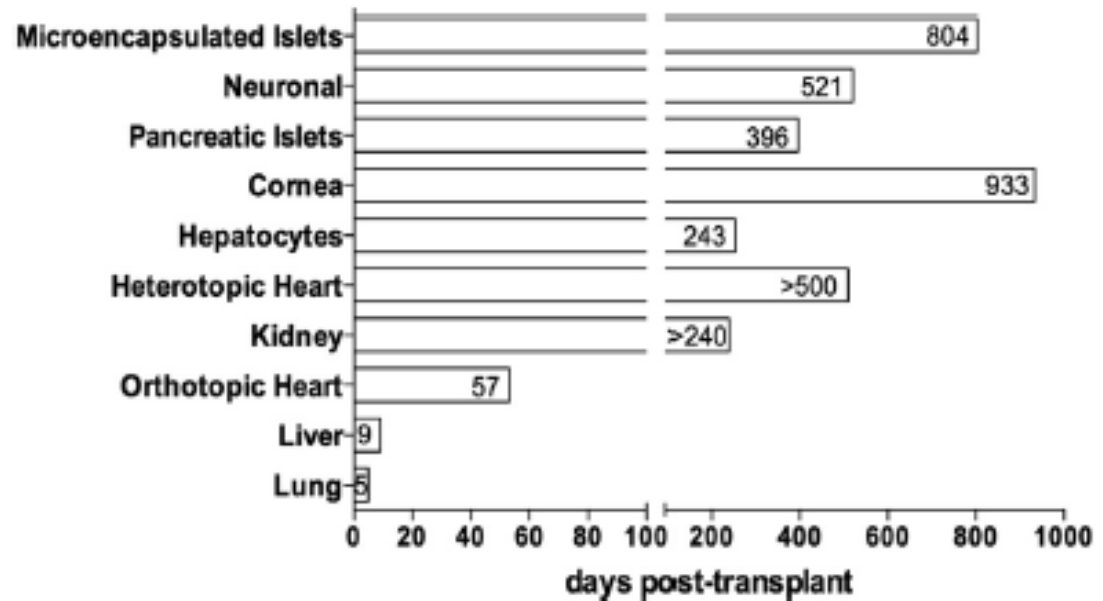


Fig. 2. Longest reported survival times of pig organ and cell xenotransplantation in preclinical trials (pig-to-nonhuman primate models). Microencapsulated pancreatic islets = 804 days (with re-Tx), 250 days (without re-Tx) (Sun et al. [35]) (wild-type [WT] pigs); Neuronal cells = >521 days (Badin et al. [31]) (CTLA4-Ig transgenic pigs); pancreatic islets = 396 days (van der Windt et al. [36]) (hCD46 pigs); cornea (full thickness) = >933 days (Choi et al. [37]) (WT pigs); hepatocytes = 243 days (with re-Tx), 80 days (without re-Tx) (Nagata et al. [38]) (WT pigs); heterotopic heart = >500 days (Mohiuddin et al. [18]) (GTKO/hCD46/hTBM pigs); kidney = >240 days (Tector AJ, personal communication) (GTKO/hCD55 pigs); orthotopic heart = 57 days (McGregor et al. [19]) (GTKO/hCD55 pigs); liver = 9 days (Kim K et al. [28]) (hCD55 pigs); lung = 5 days (Cantu et al. [29]) (vWF-deficient pigs). Figure modified from Ekser B et al [15].

Transgenic animals as organ supplier

Humanizing the pig:

Introduction of Human Genes

Knock out of pig genes \rightarrow α -1.3 galactosyltransferase \rightarrow avoid immunogenic reaction

Major Problems

Immunological Barriers

Hyperacute rejection

Acute vascular rejection

Cellular rejection

Chronic rejection

Porcine endogenous retroviruses

Comparison of kidney function between healthy humans and GTKO pigs.^a

	Human	GTKO ^b Pig
Sodium (mmol/L)	136–146	144
Potassium (mmol/L)	3.5–5.0	5.3
Chloride (mmol/L)	95–110	103.0
Calcium (mg/dL)	8.4–10.2	10.8
Phosphorus (mg/dL)	2.5–4.5	8.8
CO ₂ (mmol/L)	21–32	28.1
Urea (mg/dL)	5.0–20.0	12.8
Creatinine (mg/dL)	0.6–1.1	1.1

^a Adapted from Ekser et al. [26].

^b GTKO = α 1,3-galactosyltransferase gene-knockout.

B. Ekser et al. / International Journal of Surgery 23 (2015) 199e204

Genetically-engineered pigs produced for xenotransplantation research.

Complement regulation by human complement-regulatory gene expression:

CD46 (membrane cofactor protein)

CD55 (decay-accelerating factor)

CD59 (protectin or membrane inhibitor of reactive lysis)

Gal or nonGal antigen 'masking' or deletion:

human H-transferase gene expression (expression of blood type O antigen)

endo-beta-galactosidase C (reduction of Gal antigen expression)

α 1,3-galactosyltransferase gene-knockout (GTKO)

Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) gene-knockout (NeuGc-KO)

β 4GalNT2 (β 1,4 N-acetylgalactosaminyltransferase) gene-knockout (β 4GalNT2-KO)

Suppression of cellular immune response by gene expression or downregulation

CIITA-DN (MHC class II transactivator knockdown, resulting in swine leukocyte antigen class II knockdown)

Class I MHC-knockout (MHC-I-KO)

HLA-E/human β 2-microglobulin (inhibits human natural killer cell cytotoxicity)

human FAS ligand (CD95L)

human GnT-III (N-acetylglucosaminyltransferase III) gene

porcine CTLA4-Ig (Cytotoxic T-Lymphocyte Antigen 4 or CD152)

human TRAIL (tumor necrosis factor-alpha-related apoptosis-inducing ligand)

Anticoagulation and anti-inflammatory gene expression or deletion

von Willebrand factor (vWF)-deficient (natural mutant)

human tissue factor pathway inhibitor (TFPI)

human thrombomodulin

human endothelial protein C receptor (EPCR)

human CD39 (ectonucleoside triphosphate diphosphohydrolase-1)

Anticoagulation, anti-inflammatory, and anti-apoptotic gene expression

human A20 (tumor necrosis factor-alpha-induced protein 3)

human heme oxygenase-1 (HO-1)

Porcine asialoglycoprotein receptor 1 gene-knockout (ASGR1-KO) (decreases platelet phagocytosis)

Human signal regulatory protein α (SIRP α) (decreases platelet phagocytosis by 'self' recognition)

Prevention of porcine endogenous retrovirus (PERV) activation

PERV siRNA



Contents lists available at [ScienceDirect](#)

International Journal of Surgery

journal homepage: www.journal-surgery.net



Review

The need for xenotransplantation as a source of organs and cells for clinical transplantation



Burcin Ekser ^a, David K.C. Cooper ^{b,*}, A. Joseph Tector ^a

^a *Transplant Division, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, USA*

^b *Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA*



Contents lists available at [ScienceDirect](#)

International Journal of Surgery

journal homepage: www.journal-surgery.net



Review

Immunobiological barriers to xenotransplantation



David K.C. Cooper ^{a,*}, Burcin Ekser ^b, A. Joseph Tector ^b

^a *Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, USA*

^b *Transplant Division, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, USA*

Producing Recombinant Human Milk Proteins in the Milk of Livestock Species

Zsuzsanna Bösze, Mária Baranyi, and C. Bruce A. Whitelaw

Abstract Recombinant human proteins produced by the mammary glands of genetically modified transgenic livestock mammals represent a special aspect of milk bioactive components. For therapeutic applications, the often complex posttranslational modifications of human proteins should be recapitulated in the recombinant products. Compared to alternative production methods, mammary gland production is a viable option, underlined by a number of transgenic livestock animal models producing abundant biologically active foreign proteins in their milk. Recombinant proteins isolated from milk have reached different phases of clinical trials, with the first marketing approval for human therapeutic applications from the EMEA achieved in 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 580463, 9 pages
<http://dx.doi.org/10.1155/2013/580463>



Review Article

Expression Systems and Species Used for Transgenic Animal Bioreactors

Yanli Wang,^{1,2} Sihai Zhao,^{1,2} Liang Bai,^{1,2} Jianglin Fan,³ and Enqi Liu^{1,2}

¹ *Laboratory Animal Center, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710061, China*

² *Xi'an Jiaotong University Cardiovascular Research Center, Xi'an, Shaanxi 710061, China*

³ *Department of Molecular Pathology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi 409-3898, Japan*

Correspondence should be addressed to Enqi Liu; liuenqi@mail.xjtu.edu.cn

Received 2 November 2012; Revised 15 January 2013; Accepted 17 February 2013

Academic Editor: James D. Murray

Copyright © 2013 Yanli Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Transgenic animal bioreactors can produce therapeutic proteins with high value for pharmaceutical use. In this paper, we compared different systems capable of producing therapeutic proteins (bacteria, mammalian cells, transgenic plants, and transgenic animals) and found that transgenic animals were potentially ideal bioreactors for the synthesis of pharmaceutical protein complexes. Compared with other transgenic animal expression systems (egg white, blood, urine, seminal plasma, and silkworm cocoon), the mammary glands of transgenic animals have enormous potential. Compared with other mammalian species (pig, goat, sheep, and cow) that are currently being studied as bioreactors, rabbits offer many advantages: high fertility, easy generation of transgenic founders and offspring, insensitivity to prion diseases, relatively high milk production, and no transmission of severe diseases to humans. Noticeably, for a small- or medium-sized facility, the rabbit system is ideal to produce up to 50 kg of protein per year, considering both economical and hygienic aspects; rabbits are attractive candidates for the mammary-gland-specific expression of recombinant proteins. We also reviewed recombinant proteins that have been produced by targeted expression in the mammary glands of rabbits and discussed the limitations of transgenic animal bioreactors.

11.12.15