APPLICATION OF RABBITS IN BIOMEDICAL RESEARCH: A REVIEW

Bōsze Zs.[*], Houdebine L.M.[†]

[*]Dpt. of Animal Biology, Agricultural Biotechnology Center, Gödöllő, Hungary.
[†]Biologie du Developpement et Reproduction, Institute National de la Recherche Agronomique, Jouy en Josas, France

ABSTRACT: The first transgenic rabbits were obtained two decades ago by pronuclear microinjection. Several characteristics of rabbit made it the first and classical model for the study of lipoproteins and atherosclerosis. Rabbit models include normal cholesterol-fed rabbits, spontaneous mutants for lipid metabolism and transgenic rabbits. Though most molecular investigations of the cardiovascular system have used transgenic mice, the small rodents do not accurately reflect crucial facets of human cardiovascular physiology, therefore a number of different transgenic rabbit models of hypertrophic cardiomyopathy were created. Transgenic rabbits have been found to be suitable bioreactors for the production of pharmaceutical proteins filling an important niche between the laboratory mouse and larger farm mammals. It is the smallest animal that can be used to produce recombinant proteins in its milk or serum both on an experimental and a commercial scale. The rabbit appears particularly flexible for the preparation of human antibodies, and recombinant human proteins for replacement therapies have also been produced in rabbit milk. A specific biotechnology of the rabbit is emerging. The scientific community which uses rabbits as experimental animals or as a tool to produce biotech products, as well as those involved in breeding, are invited to focus their efforts on this species.

Key words: rabbit, human disease model, bioreactor, vaccines.

INTRODUCTION

The rabbit is a standard laboratory animal in biomedical research and transgenic rabbits are used as animal models for a variety of human diseases both genetic and acquired. Classical experimental use of rabbits includes antibody production, development of new surgical techniques, physiology and toxicity studies for the testing of new drugs.

The rabbit (*Oryctolagus cuniculus*) is phylogenetically closer to primates than are rodents (Graur *et al.*, 1996) and is large enough to permit non-lethal monitoring of physiological changes. For these reasons, a number of research groups have chosen transgenic rabbits as animal models for the study of lipoprotein metabolism, atherosclerosis, cardiovascular research and hypertrophic cardiomyopathy.

Transgenic rabbits have been found to be suitable bioreactors for the production of pharmaceutical proteins (Houdebine, 1995). The transgenic rabbit system is especially valuable because it fills an important niche between the laboratory mouse and larger farm mammals. It is the smallest animal that can be used to produce recombinant proteins in its milk or serum, both on an experimental and a commercial scale.

The first transgenic rabbits were obtained two decades ago by pronuclear microinjection (Brem *et al.*, 1985; Hammer, 1985). Since then, improvements in the methodology have been reported (Viglietta

et al., 1997, Besenfelder *et al*., 1998). This chapter summarises the specific advantages of rabbit models, the accomplishments and future perspectives for their utilisation in biomedical research.

RABBITS FOR HUMAN DISEASE MODELS

Rabbit strains have a more diverse genetic background than inbred and outbred mouse strains. This might be an advantage when studying complex disease models such as atherosclerosis or developing therapeutic strategies, since this situation mimics human genetic diversity more accurately. However, this could also be a drawback and even hamper the use of rabbits in studying the effects of gain or loss of function or in elucidating the mechanism of single gene related diseases. Though transgenic mice have become the most widely used model for human diseases, it is accepted in certain cases that the causative mutations resulting in human diseases do not give rise to the expected pathological symptoms in rodents. For example, the molecular composition of cardiac sarcomeric proteins, and therefore the mutant phenotypes, are more similar to human counterparts in rabbits than in rodent models. Moreover, a number of analytical techniques which are used to study the effects of genetic modifications in mice are limited to a single time point, since the mouse often does not survive the analysis.

Lipid metabolism and atherosclerosis

Rabbits possess several characteristics that made them the first and classical model for the study of lipoproteins and atherosclerosis. Atherosclerosis is a multifactorial disease and is one of the major causes of mortality in Western societies. Rabbit models include normal cholesterol-fed rabbits, spontaneous mutants for lipid metabolism and transgenic rabbits.

Normal rabbit strains in the study of atherosclerosis

Cholesterol fed rabbits are the classical model for studying lipid metabolism and its role in atherosclerosis, as the rabbit is extremely sensitive to a cholesterol-rich diet. Genetically defined JAX rabbits were tested for their serum cholesterol levels in response to a cholesterol-rich diet. Two rabbit strains showing five fold differences in serum cholesterol levels and different sensitivity to cholesterol have been established (Van Zutphen *et al.,* 1977). These two strains, the high responding AX/J and low responding IIIVO/J, were inbred for several generations. AX/JU and IIIVO/JU have been used for genetic analysis of quantitative traits related to dietary cholesterol susceptibility. Application of the AFLP technique with 15 primer combinations revealed 226 polymorphisms between the two inbred strains (Van Haeringen *et al*., 2002). Recent data point to the fact that dietary cholesterol induces neuronal accumulation of Alzheimer's like beta-amyloid in New Zealand White rabbits, and copper supplementation in drinking water resulted in fibrillar type beta-amyloid accumulation (Sparks, 2004).

Spontaneous mutant rabbit models of human lipoprotein disorders

Hypercholesterolemia is the most important factor in the development of atherosclerosis in humans and experimental animals. In the plasma, three kinds of atherogenic lipoproteins have been identified: low density lipoproteins (LDL) remnant lipoprotein-like **b**-very low density lipoprotein (**b**-VLDL), intermediate density lipoproteins (IDL), lipoprotein a Lp(a) and high density lipoproteins (HDL).

Watanabe heritable hyperlipidemic (WHLL) rabbits are well characterised mutants. They are deficient in LDL receptor due to a spontaneously arising deletion in exon 4 of the LDL receptor gene that encodes for a four amino acid deletion in the cysteine-rich ligand-binding domain of the protein (Yamamoto *et al*., 1986). Homozygous WHLL rabbits are hypercholesterolemic from birth and exhibit

tendon xanthoma and atherosclerosis on chow diet (Watanabe, 1980). The pathology of WHLL rabbits resembles of familiar hypercholesterolemia.

The St. Thomas rabbits have increased levels of VLDL, IDL and LDL, due to overproduction of these lipoproteins, and develop atherosclerotic lesions on a chow diet (LaVille *et al.*, 1987). The St Thomas Hospital rabbit strain is thus a potential model for familiar combined hyperlipidemia.

Transgenic rabbits for the study of atherosclerosis

Compared with the most widely used transgenic model, which is the mouse, rabbits have different lipoprotein metabolism features, as summarised in Table 1. Ideally the animal models should share the most important characteristics of the disease with man, which is the case with rabbits. The first transgenic rabbits used to study lipid metabolism by overexpressing the human hepatic lipase and the human apolipoprotein A1 genes were obtained in 1994 (Fan *et al.*, 1994; Duverger *et al.*, 1994). Since then, an increasing number of genetically engineered rabbits have become tools in exploring the function of the proteins associated with dyslipidemia and atherosclerosis (Table 2).

To date, more than a dozen transgenes have been expressed in transgenic rabbits. Some of these animals were naturally mated with each other or with WHLL rabbits to create double mutant animals. The phenotypic properties of transgenic rabbit models in the study of atherosclerosis and dyslipidemia have been described (Brousseau and Hoeg, 1999; Fan *et al.,* 1999; Fan and Watanabe, 2000a; 2003).

In some cases, several genes were introduced simultaneously. This situation was encountered for the rabbits harbouring the human apoA1-apoCIII-apoAIV gene cluster, which allows a coordinated expression of the three genes (Recalde *et al* 2004). Other transgenic rabbits not described here are also used to study lipid metabolism and obesity (unpublished data).

These animals are being used to evaluate the effects of new drugs which stimulate human genes and are expected to protect patients against atherosclerosis. These rabbits were fed with a cholesterol-

Table 2: Application of transgenic rabbit models in lipid metabolism and atherosclerosis.

Expressed human gene	Main results	Reference(s)
Human hepatic lipase	Key role in plasma cholesterol homeostasis. Elevated hepatic lipase levels inhibited diet-induced atherosclerosis.	Fan et al. (1994)
Human lipoprotein lipase	Reduced total cholesterol and atherogenic very low density lipoprotein levels. 80% decrease in plasma triglycerides 59% decrease in high density lipoprotein-cholesterol.	Araki et al. (2000) Fan et al. (2001)
Human 15-lipoxygenase	Protection against atherosclerosis development.	Shen et al. (1995)
Human lecithin:cholesterol acyltransferase	Significantly less aortic atherosclerosis.	Hoeg et al. (1996)
Human apo A-I	Inhibition of atherosclerosis, impaired vasorelaxation. Isolated increase in high density cholesterol levels not effective in inhibiting atherosclerosis. Pro-atherosclerotic diet equally reduced paroxonase activity in control and apo A-I transgenic rabbits.	Duverger et al. (1996a, b) Lebuffe et al. (1997) Boullier et al. (2001) Mackness et al. (2000)
Human apo A	Covalent association with rabbit apo B, new insights into Lp(a) assembly. Extensive atherosclerosis in apo A transgenic vs. normal rabbits in response to cholesterol rich diet.	Rouy et al. (1998) Fan et al. (2000) Fan and Watanabe (2000b)
Human apo B-100	Overexpression of apo B resulted increased levels of the atherogenic low density lipoprotein (LDL) and decreased levels of the anti-atherogenic high density lipoproteins (HDL).	Fan et al. (1995)
Human apoA-I/C-III/A-IV gene cluster	Higher plasma total cholesterol, HDL-cholesterol and total phospholipid concentrations.	Recalde et al. (2004)
Human apo E variants apo E2 apo E3	Animal model of type III hyperlipoproteinemia, points to the role of sex hormones. Reduced levels of very low density lipoprotein, accumulation of low density lipoprotein.	Huang et al. (1997) Fan et al. (1998)
Macrophage derived Human matrix metalloproteinase 12	Animal model for mechanistic studies in inflammatory diseases and cancer invasion. Enhanced arthritic lesions and cartilage degeneration.	Fan et al. (2004) Wang et al. (2004)
Macrophage derived Lipoprotein lipase	Macrophage derived lipoprotein lipase is proatherogenic.	Ichikawa et al. (2005)
Rabbit apo B mRNA editing enzyme APOBEC-1 component 1 (APOBEC-1)	Liver displasia in all transgenic rabbit lines => not applicable for gene therapy. Due to low expression levels apo B mRNA is not edited. The distinct CpG methylation pattern of the rat proximal promoter is lost in the transgenic rabbit	Yamanaka et al. (1995) Apostel et al. (2002)

4

On the other hand, in a number of cases transgenic rabbits showed different phenotypes from transgenic mice, even though the same genes were introduced and the phenotype of transgenic rabbits was closer to humans (Fan and Watanabe, 2003). This again underlines the importance of choosing an appropriate species to be used in transgenic investigation.

Hypertrophic cardiomyopathy

*In vi*vo models are invaluable to provide integrative data regarding physiological and pathological

AIDS, cancer and spongiform encephalopathy studies

Animal models are essential for understanding the pathogenesis of retrovirus-induced immune deficiency and encephalopathy and for development and testing of new therapies and vaccines. AIDS and related disorders are etiologically linked to various members of the lentivirus subfamily of retroviruses. These lymphocytopathic lentiviruses are designated human immuno-deficiency virus type 1 (HIV-1) and human immuno-deficiency virus type 2 (HIV-2). The only animals susceptible to experimental HIV-1 infection are the chimpanzee, the gibbon ape, and the rabbit. The progression of HIV-1 infection in laboratory rabbits is very slow due to critical differences between human and rabbit CD4 binding sites for viral protein HIV gp120. To tentatively improve this model, transgenic rabbits that express the human CD4 gene were created (Snyder *et al.,* 1995, Dunn *et al.,* 1995). *In vitro* data on transgenic rabbit lymphocytes revealed the requirement for huCD4 in initiation, but not the spread of HIV-1-induced apoptosis (Leno *et al.,* 1995). In vivo infection of huCD4-transgenic rabbits using HIV-1IIIB-infected autologous lymphocytes was demonstrated by virus isolation, detection of HIV-1-specific DNA in peripheral blood lymphocytes and seroconversion to various HIV-1 proteins (Dunn *et al.,* 1995). In spite of the promising results obtained with the huCD4 transgenic rabbits, which were created by two independent laboratories, no additional data based on utilisation of the huCD4 transgenic rabbits were published. It would have been interesting to sensitize the CD4 rabbits to HIV by adding the human CCR5 gene, which is the second essential component of HIV receptor. These experiments were not carried out mainly because rabbit handling at level 3 confinement is too costly.

Oncogene expressing transgenic rabbits, which developed lymphomas (Knight *et al*., 1988, Sethupathi *et al.,* 1994) and skin carcinoma (Peng *et al*., 1993; 1995; 1999; 2001) were created and partially characterised. Those animal models may be valuable for studying oncogenes and their synergistic effects in tumorigenesis. Furthermore they provide a model for evaluating antitumor therapies (reviewed by Fan and Watanabe, 2003; Bõsze *et al.,* 2003).

Transgenic rabbits expressing the ovine PrP gene have been obtained. These animals, but not the non-transgenic control, proved highly sensitive to infection by scrapie (unpublished data). These rabbits are useful models for deciphering the mechanisms of prion infection.

Anatomical and metabolic disorders

Acromegaly is a chronic disease in which prolonged elevation of growth hormone (GH) levels after puberty leads to overgrowth of the skull and of the extremities. Glucose intolerance and insulin resistance is manifested in about 60% of the patients (Ganda and Simonson 1993). GH overexpressing transgenic mice had giant phenotype, which is not a relevant model for human acromegaly (Palmiter *et al*., 1982). Trangenic pigs overexpressing GH did not show typical acromegalic alterations, but developed arthritis and ulcers (Pinkert *et al.,* 1994). On the other hand, transgenic rabbits expressing GH gene reiterated the skeletal abnormalities, the metabolic differences and the histopathological alterations in the liver and muscle of humans suffering from acromegaly (Costa *et al.,* 1998).

Surgically induced secondary osteoarthritis and osteotomy rabbit models have also been created for *in vivo* gene therapy trials (Rogart *et al.,* 1999, Bertone *et al.,* 2004). In the osteoarthritis model, the interleukin-1 receptor antagonist gene was found to be effective in the prevention of osteoarthritis progression (Fernandes *et al*., 1999). In the osteotomy model the gene therapy vector harboured the human BMP-6 gene and was shown to accelerate healing (Bertone *et al*., 2004).

Transgenic rabbit organs expressing human DAF and CD59 genes in all their cells are resistant to complement attack (Taboit-Dameron *et al*., 1999).

Use of GFP rabbits

GFP (green fluorescent protein) is a protein from Pacific Ocean jellyfish which becomes green under UV light. It is increasingly used as a marker to follow cells expressing the GFP gene. One advantage of this protein is that its fluorescence needs no substrate and that repeated non-invasive observations can be performed *in vivo* on the same animal.

Various transgenic species, including rabbits, harbouring the GFP gene have been obtained. The human eF1-**a** gene promoter was used to drive the expression of GFP gene in all cell types of the rabbits (Boulanger *et al*., 2002).

The observation of the GFP rabbit cells using a specific probe (Cell-viZio, MKT) made it possible to obtain the precise description of different tissues which appear green (Al Gubory and Houdebine, unpublished data).

GFP rabbits are currently being used as models to study cornea grafting. These rabbits made following the fate of the green cells possible at different periods after cornea grafting on normal rabbits (Cardiatikis-Myers *et al*., unpublished data).

TRANSGENIC RABBITS AS BIOREACTORS

Pharmaceutical proteins produced from transgenic rabbit milk

The technology for using the mammary gland as a bioreactor has been developed to the point that pharmaceuticals derived from the milk of transgenic farm animals are currently in the advanced stages of clinical trials. It is generally admitted that transgenic organisms may be the source of abundant and cheap therapeutic proteins. Transgenic plants suffer from the fact that they do not glycosylate proteins as animal cells do. Moreover, no dissemination of transgenic plants synthesizing pharmaceutical proteins should occur. Animals do not raise these two problems (Houdebine, 2002).

The time required to generate transgenic animals with high expression levels and to deliver a product to the market are the major drawbacks to large animal transgenic technology. Transgenic rabbits are an attractive alternative to large dairy animals because of their large litter size and short generation interval (Houdebine, 1995; Dove, 2000). Rabbits are easily milked and their milk composition is well characterised (Baranyi *et al*. 1995). Rabbit milk naturally contains 2.5 and 4.8 times as much protein as sheep and goat milk respectively (Jennes *et al*., 1994). The transgenic rabbit is a very efficient system for testing new gene constructs and has the advantage over mice that sufficient protein can be produced for activity testing and even for clinical trials of therapeutic proteins (Van den Hout *et al*., 2001, 2004). Pharmaceutical products which were produced in transgenic rabbit milk can be divided into three categories: (a) hormones and bioactive peptides, (b) therapeutic proteins and (c) vaccines. Table 3 shows the hormones and bioactive peptides which have been produced in transgenic rabbit milk to date and indicates the observed side-effects of the recombinant proteins on animals. On the other hand, it should be considered that one of the theoretical concerns, and the reason for the moderate public acceptance in some countries for the production of bioactive hormones from the milk of transgenic animals, is their potential deleterious side-effects on reproducing-females and their litters.

Recombinant human proteins for replacement therapies have also been produced in rabbit milk. To date, production of human C1 inhibitor, alpha-glucosidase and the blood clotting factor VIII were reported (Bijvoet *et al*., 1999, Hiripi *et al*., 2003, Koles *et al.,* 2004a). Among these proteins, the human C1 inhibitor and alpha-glucosidase reached the phase III clinical trials (http:// www.pharming.com/, Van den Hout, 2004).

Table 3: Hormones and bioactive peptides produced in the milk of transgenic rabbits.

Expressed protein	Gene	Maximal expression levels Other findings/side-effects	Reference(s)
Interleukin 2 (IL29)	Rabbit **b**-casein-genomic DNA	0.0005 g/l	Buhler et al., 1990
Human growth hormone	Mouse whey acidic protein-genomic DNA	0.05 g/l hGH in the serum.	Limonta et al., 1995
Human insulin-like growth factor	Bovine **a**s1-casein expression casette-cDNA	1 g/l. Upregulation of IGFB-2, increased milk yield.	Brem et al., 1994; Wolf et al., 199; Zinovieva et al., 1998
Bovine chymosin	Bovine **a**s1-casein expression cassette prochymosin gene	10 g/l. Healthy animals and no unwanted clotting.	Brem et al., 1995
Erythropoietin	Rabbit whey acidic protein-cDNA	0.0003 g/l	Rodriguez et al., 1995
Erythropoietin	Rabbit whey acicic protein-cDNA and genomic DNA	0.05 g/l High red blood cell counts, sterility, agalactia and premature death in one transgenic line.	Massoud et al., 1996
EC superoxide dismutase	Mouse whey acidic protein-cDNA EC SOD	3 g/l. Not fully glycosylated, biologically active.	Strömqvist et al., 1996
Erythropoietin	Bovine **b**-lactoglobulin-cDNA (fusion protein of erythropoietin with **b**-lactoglobulin to avoid biological activity in the secreting animal)	0.05 g/l. Correct glycosylation, transient increase in hematocrit values of lactating females.	Korhonen et al., 1997
Salmon calcitonin	Ovine **b**-lactoglobulin-cDNA (fusion protein of salmon calcitonin with human -lactoglobulin)	2.1 g/l. First evid.4(88 2.3n g)71.c8 2.3(n d.4(8f))-89.9( )-14.C-(r)-33.8(t)32.8 2.34(r	

Though the number of applications is steadily increasing, it should be pointed out that the commercial success of recombinant hormones, bioactive peptides, therapeutic proteins or vaccines which are in competition with alternative methods of production has still not occurred. One of the reasons is that proteins are naturally very complex molecules and that transgenic animals are a new method of producing these pharmaceuticals. This means that the pioneer molecules obtained in this way are very closely examined before being released on the market.

bispecific protein, which was purified from the serum of the transgenic rabbits was directed to a melanoma-associated proteoglycan and the human CD28 molecule. It was fully active and mediated target-cell restricted T cell stimulation and tumor cell killing.

Polyclonal antibodies

Though monoclonal human-like antibodies currently represent a market of $9 billion yearly sales, they react with specific targets, which may change, evolve and mutate during the spread of disease throughout a population or within an individual. Polyclonal antibodies are highly potent against multiple antigenic targets, can neutralize toxins and direct immune responses to eliminate pathogens. Since rabbits have been used for many decades for the commercial production of polyclonal antibodies, an early-stage biopharmaceutical company, the Therapeutic Human Polyclonals Inc (http://www.polyclonals.com/) started developing transgenic rabbits producing human polyclonal antibodies after conventional immunisation of the animals with the antigens of interest. The proof of principle experiments was presented recently by Buelow (2006). The same project is being developed also in mice, pigs and cows. It should be mentioned that trans-chromosomic calves carrying the entire human immunoglobulin (hIg) heavy-chain (H) and lambda (lambda) light-chain loci represent another transgenic livestock species to produce polyclonal human antibodies (Kuroiwa *et al.,* 2002).

To obtain fully human polyclonal antibodies, the human loci containing the gene coding for antibodies must be introduced into the genome of the animals, which must also have lost their own immunoglobulin genes. This can be achieved in mice by knocking out immunoglobulin genes in ES cells and generating chimaeric animals harbouring the mutation (Ishida *et al*., 2002). In other species, gene knockout must be performed in somatic cells further used to generate cloned mutated animals (Kuroiwa *et al.,* 2002).

Available rabbit strains are naturally devoid of several immunoglobulin loci. These animals, which received human immunoglobulin loci, generate human polyclonal antibodies after classical immunisation (Buelow, 2006). The rabbit appears to be particularly flexible in the preparation of human antibodies.

Vaccines

Conventional vaccines rely on the use of attenuated pathogens. These vaccines are often efficient but not always fully safe and not easily prepared in large quantities. Recombinant pathogen proteins appear to be getting safer and more easily available than classical vaccines.

Associated viral proteins forming VLPs (virus like particles) having strong immunogenic activities and being devoid of nucleic acids proved to be efficient vaccines in several cases. VLPs containing VP2 and VP6 rotavirus proteins have been obtained in rabbit milk at a concentration as high as 500µg/ml (http://www.bioprotein.com). Milk fractions administered in mice protected 100% of the animals against an infection by rotavirus (Soler *et al*., 2005; 2006)

CONCLUSIONS

The experiments described in this paper and others emphasize the specific importance of the rabbits as models and protein producers. Rabbits are also a significant source of meat in different countries. This supports the idea that the rabbit will be more widely used for specific biotechnology projects. Transgenesis is presently a limited but classical tool in this species. Cloning has been successfully achieved, although this technique must be improved to be really utilisable in creating valuable models. Rabbit genome sequencing is in the course of providing researchers with unknown genes

and genetic markers. A clear limitation for the use of rabbits as models in some cases is their extreme genetic diversity. The establishment of one or two lines of inbred rabbits having more homogeneous biological properties would be very useful.

A specific biotechnology of the rabbit is therefore emerging. The first international meeting on transgenic rabbits took place in Tsukuba (Japan) in 2005. Another meeting on "Use of rabbits as models of infectious diseases in humans" was also held in 2005 at the NIH, USA. The scientific community which uses rabbits as experimental animals or as a tool for the production of biotech products, as well as those involved in breeding, would be well advised to focus their efforts on this species.

REFERENCES

Apostel F., Dammann R., Pfeifer G.P., Greeve J. 2002. Reduced expression and increased CpG dinucleotide methylation of the rat APOBEC-1 promoter in transgenic rabbits. *Biochim Biophys Acta., 1577, 384-94.*

Araki M., Fan J.L., Challah M., Bensaudon A., Yamada N., Honda K., 2000. Transgenic rabbits expressing human lipoprotein lipase C*ytotechnology, 33, 93-99.*

Baranyi M., Brignon G., Anglage P., Ribadeau-Dumas B. 1995. New data on the proteins of rabbit (*Oryctolagus cuniculus*) milk. *Comp Biochem Physiol B Biochem Mol Biol., 111, 407-15.*

Bertone A.L., Pittman D.D., Bouxsein M.L., Li J., Clancy B., Seeherman HJ. 2004. Adenoviral-mediated transfer of human BMP-6 gene accelerates healing in a rabbit ulnar osteotomy model. *J Orthop Res., 22, 1261-70.*

Besenfelder U., Aigner B., Müller M., Brem G. 1998. Generation and application of transgenic rabbits. In: *Microinjection and transgenesis* A. Cid-Arregui and A.Garcia-Carrancám, eds. (Springer-Verlag, Berlin) *pp.* 561-586.

Bijvoet A.G., Van Hirtum H., Kroos M.A., Van de Kamp E.H., Schoneveld O., Visser P., Brakenhoff J.P., Weggeman M., van Corven E.J., Van der Ploeg A.T., Reuser AJ. 1999. Human acid alpha-glucosidase from rabbit milk has therapeutic effect in mice with glycogen storage disease type II. *Hum Mol Genet., 8, 2145-53.*

Boulanger L., Mallet S., Chensé P., Chrenek P., Viglietta C., Houdebine L.M., Renard J.P. 2002. Advantages and limits of using the ubiquitous expressed EF1alpha promoter for transgenesis in vivo and in vitro in rabbit. *Transgenic Res., 11, 88.*

Boullier A., Hennuyer N., Tailleux A., Furman C., Duverger N., Caillaud J.M., Castro G., Fievet C., Fruchart J.C., Duriez P. 2001. Increased levels of high-density lipoprotein cholesterol are ineffective in inhibiting the development of immune responses to oxidized low-density lipoprotein and atherosclerosis in transgenic rabbits expressing human apolipoprotein (apo) A-I with severe hypercholesterolaemia. *Clin Sci (Lond)., 100, 343-55.*

Bõsze Z., Hiripi L., Carnwath J.W., Niemann H. 2003. The transgenic rabbit as model for human diseases and as a source of biologically active recombinant proteins. *Transgenic Res., 12, 541-53.*

Brem G., Brenig B., Godman H.M., Selden R.C., Graf F., Kruff B., Springmann K., Hondele J., Meyer J., Winnacker E.-L., Krauszlich H. 1985. Production of transgenic mice, rabbits and pigs by microinjection into pronuclei. *Zuchthygiene 20, 251-252.*

Brem G., Hartl P., Besenfelder U., Wolf E., Zinovieva N., Pfaller R. 1994. Expression of synthetic cDNA sequences encoding human insulin-like growth factor-1 (IGF-1) in the mammary gland of transgenic rabbits. *Gene., 149, 351-5.*

Brem G., Besenfelder U., Zinovieva N., Seregi J., Solti L., Hartl P. 1995. Mammary gland-specific expression of chymosin constructs in transgenic rabbits. *Theriogenology, 43, 175.*

Brousseau M.E., Hoeg J.M. 1999. Transgenic rabbits as models for atherosclerosis research. *J. Lipid Res., 40, 365-75.*

Buelow R. (2006) Expression of a humanized antibody repertoire in transgenic rabbits. Conference V. Tahoe City USA. *Transgenic Res., 15, 120.*

Buhler T.A., Bruyere T., Went D.F., Stranzinger G., Burki K. 1990. Rabbit beta-casein promoter directs secretion of human interleukin-2 into the milk of transgenic rabbits. *Biotechnology (N Y)., 8, 140-3.*

Costa C., Solanes G., Visa J., Bosch F. 1998. Transgenic rabbits overexpressing growth hormone develop acromegaly and diabetes mellitus. *FASEB J., 12, 1455-60.*

Coulibaly S., Besenfelder U., Fleischmann M., Zinovieva N., Grossmann A., Wozny M., Bartke I., Togel M., Muller M., Brem G. 1999. Human nerve growth factor beta (hNGF-beta): mammary gland specific expression and production in transgenic rabbits. *FEBS Lett. 444, 111-6.*

Coulibaly S., Besenfelder U., Miller I., Zinovieva N., Lassnig C., Kotler T., Jameson J.L., Gemeiner M., Muller M., Brem G. 2002. Expression and characterization of functional recombinant bovine follicle-stimulating hormone (boFSHalpha/beta) produced in the milk of transgenic rabbits. *Mol Reprod Dev., 63, 300-8.*

Dorman S.E., Hatem C.L., Tyagi S., Aird K., Lopez-Molina J., Pitt M.L., Zook B.C., Dannenberg A.M. Jr, Bishai W.R., Manabe Y.C. 2004. Susceptibility to tuberculosis: clues from studies with inbred and outbred New Zealand White rabbits. *Infect Immun., 72, 1700-5.*

Dove A. 2000. Milking the genome for profit. *Nature Biotechnol., 18, 1045-1048.*

Dunn C.S., Mehtali M., Houdebine L.M., Gut J.P., Kirn A., Aubertin A.M., 1995. Human immunodeficiency virus type 1 infection of human CD4-transgenic rabbits. *J Gen Virol., 76, 1327-36.*

Duverger N., Emmanuel F., Viglietta C., Tailleux A., Benoît P., Atternot F., Cuine S., Fievet C., Fruchard J.C., Houdebine L.M., Denefle P. 1994. Transgenic rabbits expressing human apolipoprotein A-I in the liver. *Thrombosis and Vasc. Biol., 90, 1-240.*

Duverger N., Kruth H., Emmanuel F., Caillaud J.M., Viglietta C., Castro G., Tailleux A., Fievet C., Fruchart J.C., Houdebine L.M., Denefle P. 1996a Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A-I-transgenic rabbits. *Circulation, 94, 713-7.*

Duverger N., Viglietta C., Berthou L., Emmanuel F., Tailleux A., Parmentier-Nihoul L., Laine B., Fievet C., Castro G., Fruchart J.C., Houbebine L.M., Denefie P. 1996b Transgenic rabbits expressing human apolipoprotein A-I in the liver. *Arterioscler Thromb Vasc Biol., 16, 1424-9.*

Edmunds T., Van Patten S.M., Pollock J, Hanson E., Bernasconi R., Higgins E., Manavalan P., Ziomek C., Meade H., McPherson J. M., and Cole E.S. 1998. Transgenically Produced Human Antithrombin: Structural and Functional Comparison to Human Plasma-Derived Antithrombin. *Blood, 12, 4561-4571.*

Fan J., Watanabe T. 2000a. Cholesterol-fed and transgenic rabbit models for the study of atherosclerosis. *J Atheroscler Thromb., 7, 26-32.*

Fan J., Watanabe T. 2000b Transgenic rabbits expressing human apolipoprotein (a). *J Atheroscler Thromb., 7, 8-13.*

Fan J., Watanabe T. 2003. Transgenic rabbits as therapeutic protein bioreactors and human disease models. *Pharmacol Ther., 99, 261-82.*

Fan J., Wang J., Bensadoun A., Lauer S.J., Dang Q., Mahley R.W., Taylor J.M. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc Natl Acad Sci U S A., 91, 8724-8.*

Fan J., McCormick S.P., Krauss R.M., Taylor S., Quan R., Taylor J.M., Young S.G. 1995. Overexpression of human apolipoprotein B-100 in transgenic rabbits results in increased levels of LDL and decreased levels of HDL. *Arterioscler Thromb Vasc Biol., 15, 1889-99.*

Fan J., Ji Z.S., Huang Y., de Silva H., Sanan D., Mahley R.W., Innerarity T.L., Taylor J.M. 1998. Increased expression of apolipoprotein E in transgenic rabbits results in reduced levels of very low density lipoproteins and an accumulation of low density lipoproteins in plasma. *J Clin Invest., 101, 2151-64.*

Fan J., Challah M., Watanabe T. 1999. Transgenic rabbit models for biomedical research: current status, basic methods and future perspectives. *Pathol Int., 49, 583-94.*

Fan J., Challah M., Shimoyamada H., Watanabe T. 2000. Transgenic rabbits expressing human apolipoprotein(a) as a useful model for the study of lipoprotein(a). *Ann N Y Acad Sci., 902, 347-51.*

Fan J., Unoki H., Kojima N., Sun H., Shimoyamada H., Deng H., Okazaki M., Shikama H., Yamada N., Watanabe T. 2001. Overexpression of lipoprotein lipase in transgenic rabbits inhibits diet-induced hypercholesterolemia and atherosclerosis. *J Biol Chem., 276, 40071-9.*

Fan J., Wang X., Wu L., Matsumoto S.I., Liang J., Koike T., Ichikawa T., Sun H., Shikama H., Sasaguri Y., Watanabe T. 2004. Macrophage-specific overexpression of human matrix metalloproteinase-12 in transgenic rabbits. *Transgenic Res., 13, 261-9.*

Fernandes J., Tardif G., Martel-Pelletier J., Lascau-Coman V., Dupuis M., Moldovan F., Sheppard M., Krishnan B.R., Pelletier J.P. 1999. In vivo transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: prevention of osteoarthritis progression. *Am J Pathol., 154, 1159-69.*

Galet C., Le Bourhis C.M., Chopineau M., Le Griec G., Perrin A., Magallon T., Attal J., Viglietta C., Houdebine L.M. and Guillou F. 2001. Expression of a single beta-alpha chain protein of equine LH/CG in milk of transgenic rabbits and its biological activity. *Mol. Cell. Endocrinol., 174, 31-40.*

Ganda O.P., Simonson D. C. 1993. Growth hormone, acromegaly and diabetes. *Diabetes Res., 1, 286-300.*

Graur D., Duret L., Gouy M. 1996. Phylogenetic position of the order Lagomorpha (rabbits, hares and allies) *Nature., 379, 333-335.*

Groose- Hovest L., Muller S., Minoia R., Wolf E., Zakharchenko V., Wenigerkind H., Lassnig C., Besenfelder U., Muller M., Lytton S.D., Jung G., Brem G. 2004. Cloned transgenic farm animals produce a bispecific antibody for T cell-mediated tumor cell killing. *Proc Natl Acad Sci U S A. 101, 6858-63.*

Hammer R.E., Pursel V.G., RexrMAodR.E Jr̞l Wllr R J.,Boltr D J.,Ebert, . , B i n s t m e r R L M . 1 8 5 6 . p r o d u transgenic rabbit, sheeps andpigts yn macrinjeuitiog*Natur.,*

H o u d e b
fcrof thn mil

i n B i t e c h
uaincYV.-., chwend

I(.,)-17521Ty80

producing human immunoglobulin. *Nat Biotechnol., 20, 889-94*.

La Ville A., Turner P.R., Pittilo R.M., Martini S., Marenah C.B., Rowles P.M., Morris G., Thomson G.A., Woolf N., Lewis B. 1987. Hereditary hyperlipidemia in the rabbit due to overproduction of lipoproteins. I. Biochemical studies. *Arteriosclerosis, 7, 105-12*.

Lebuffe G., Boullier A., Tailleux A., Delfly B., Dupuis B., Fruchart

Johanson T., Viglietta C., Puissant C., Hannson L. 1996. Recombinant human extracellular superoxide dismutase produced in milk of transgenic rabbits. *Transgenic Res., 6, 271-278.*

Subramanian A., Paleyanda R.K., Lubon H., Williams B.L., Gwazdauskas F.C., Knight J.W., Drohan W.N., Velander W.H. 1996. Rate limitations in posttranslational processing by the mammary gland of transgenic animals. *Ann N Y Acad Sci., 782, 87-96.*

Taboit-Dameron F., Malassagne B., Viglietta C., Puissant C., Leroux-Coyau M., Chereau C., Attal J., Weill B. and Houdebine L.M. 1999. Association of the 5'HS4 sequence of the chicken beta-globin locus control region with human EF1 alpha gene promoter induces ubiquitous and high expression of human CD55 and CD59 cDNAs in transgenic rabbits. *Transgenic Res., 8, 223-235.*

Tardiff J.C., Factor S.M., Tompkins B.D., Hewett T.E., Palmer B.M., Moore R.L., Schwartz S., Robbins J., Leinwand L.A. 1998. A truncated cardiac troponin T molecule in transgenic mice suggests multiple cellular mechanisms for familial hypertrophic cardiomyopathy. *J Clin Invest., 101, 2800-11.*

Van den Hout J.M., Reuser A.J., de Klerk J.B., Arts W.F., Smeitink J.A., Van der Ploeg A.T. 2001. Enzyme therapy for pompe disease with recombinant human alpha-glucosidase from rabbit milk. *J Inherit Metab Dis., 24, 266-74.*

Van den Hout J.M., Kamphoven J.H., Winkel L.P., Arts W.F., De Klerk J.B., Loonen M.C., Vulto A.G., Cromme-Dijkhuis A., Weisglas-Kuperus N., Hop W., Van Hirtum H., Van Diggelen O.P., Boer M., Kroos M.A., Van Doorn P.A., Van der Voort E., Sibbles B., Van Corven E.J., Brakenhoff J.P., Van Hove J., Smeitink J.A., de Jong G., Reuser A.J., Van der Ploeg A.T. 2004. Long-term intravenous treatment of Pompe disease with recombinant human alpha-glucosidase from milk. *Pediatrics, 113, e448-57.*

Van Haeringen W.A., Den Bieman M.G., Lankhorst A.E., van Lith H.A., van Zutphen L.F. 2002. Application of AFLP markers for QTL mapping in the rabbit. *Genome, 45, 914-21.*

Van Zutphen L.F., Fox R.R. 1977. Strain differences in response to dietary cholesterol by JAX rabbits: correlation with esterase patterns. *Atherosclerosis, 28, 435-46.*

Viglietta C., Massoud M., Houdebine L.M. 1997. The generation of transgenic rabbits. In *Transgenic Animals ed. Houdebine L.M. Harwood Academic Publishers pp., 3/1-3/3.*

Wang X., Liang J., Koike T., Sun H., Ichikawa T., Kitajima S., Morimoto M., Shikama H., Watanabe T., Sasaguri Y., Fan J. 2004. Overexpression of human matrix metalloproteinase-12 enhances the development of inflammatory arthritis in transgenic rabbits. *Am J Pathol., 165, 1375-83.*

Watanabe Y. 1980. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). *Atherosclerosis, 36, 261-268.*

Weidle U.H., Lenz H., Brem G. 1991. Genes encoding a mouse monoclonal antibody are expressed in transgenic mice, rabbits and pigs. *Gene., 98, 185-91.*

Wolf E., Jehle P.M., Weber M.M., Sauerwein H., Daxenberger A., Breier B.H., Besenfelder U., Frenyo L., Brem G. 1997. Human insulin-like growth factor I (IGF-I) produced in the mammary glands of transgenic rabbits: yield, receptor binding, mitogenic activity, and effects on IGF-binding proteins. *Endocrinology, 138, 307-13.*

Yamamoto T., Bishop R.W., Brown M.S., Goldstein J.L., Russell D.W. 1986. Deletion in cysteine-rich region of LDL receptor impedes transport to cell surface in WHHL rabbit. *Science, 232, 1230-1237.*

Yamanaka S., Balestra M.E., Ferrell L.D., Fan J., Arnold K.S., Taylor S., Taylor J.M., Innerarity T.L.1995. Apolipoprotein B mRNA-editing protein induces hepatocellular carcinoma and dysplasia in transgenic animals. *Proc Natl Acad Sci U.S.A. 92, 8483-7.*

Zinovieva N., Lassnig C., Schams D., Besenfelder U., Wolf E., Muller S., Frenyo L., Seregi J., Muller M., Brem G. 1998. Stable production of human insulin-like growth factor 1 (IGF-1) in the milk of hemi- and homozygous transgenic rabbits over several generations. T*ransgenic Res., 7, 437-47.*